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A MANUAL
OF
BACTERIOLOGY

CLINICAL AND APPLIED

BY

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THIRD EDITION



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PREFACE

TO

THE THIRD EDITION

In this Third Edition, while the general arrangement of the contents remains the same as before, the text has been subjected to complete revision, much new matter has been incorporated, and many sections have been re-written. Several illustrations have been added, and for the new photo-micrographs I have to thank Dr. HURRY, Bacteriological Laboratory, King's College. I am indebted to my friend and colleague Dr. FRANK TAYLOR, Lecturer on Bacteriology in King's College, for many suggestions and for much assistance in the revision of the proof-sheets.

R. T. H.

KING'S COLLEGE, LONDON:
October, 1908.

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PREFACE

TO

THE SECOND EDITION

In this new edition the text has been revised throughout, several sections have been re-written, and the whole work has, it is hoped, been brought up to date. Some slight re-arrangement of the contents has also been made, and the majority of the photo-micrographs, many of which are new, have been transferred from the text to plates. For valuable suggestions in the section on Malaria I am indebted to Dr. DANIELS, Superintendent of the London School of Tropical Medicine, while in the revision of the proof-sheets I have been greatly helped by Dr. H. S. WILLSON and Mr. F. J. TANNER. Mr. J. E. BARNARD and Mr. FRANK STRATTON have again contributed the new photo-micrographs and drawings respectively.

KING'S COLLEGE, LONDON:

July, 1902.

PREFACE

In the following manual I have endeavoured to give some account of those portions of Bacteriology which are of especial interest in clinical medicine and hygiene. The preparation of tissues, methods of culture, descriptions of pathogenic organisms and their detection, the examination of water, &c., have therefore been given at some length. As it would be impossible in the space at my disposal to include everything relative to the subject, a selection has had to be made, and such details as the celloidin method, Löffler's stain for flagella, the strictly animal parasitic diseases (with a few exceptions), &c., have, among others, been omitted.

At the end of the sections dealing with the pathogenic organisms which attack man, some directions have been given for the bacteriological clinical diagnosis and examination, but these are in no way exhaustive; in fact, it would not be possible in a short work to give a scheme of examination which would cover every case. These directions will also render the book of service in the laboratory, while I venture to hope that the details given in the Appendix on the use of the remedies and diagnostic agents of bacterial origin may be of value to the practitioner.

MR. F. FOULERTON. My thanks are
and to Mr. FRANK STRATTON respectively
graphs and original drawings, while for
illustrations blocks have been kindly let
and TATLOCK, and Messrs. SWIFT & SON

May, 1898.

CONTENTS

CHAPTER	PAGE
INTRODUCTION	1
I. THE NATURE, STRUCTURE, AND FUNCTIONS OF THE BACTERIA: THEIR CLASSIFICATION, GENERAL BIOLOGY, AND CHEMISTRY—BACTERIA AND DISEASE	8
II. METHODS OF CULTIVATING AND ISOLATING ORGANISMS	37
III. THE PREPARATION OF TISSUES AND ORGANISMS FOR STAINING AND MOUNTING—STAINING AND STAINING METHODS	76
IV. METHODS OF INVESTIGATING MICROBIAL DISEASES—THE INOCULATION AND DISSECTION OF ANIMALS—HANGING-DROP CULTIVATION—INTERLAMELLAR FILMS—THE MICROSCOPE	109
V. INFECTION—VEGETABLE AND ANIMAL PARASITES—THE INFECTIVE PROCESS—ANTI-BODIES—ANTI-SERA AND ANTITOXINS—IMMUNITY	184
VI. SUPPURATION AND SEPTIC CONDITIONS	200
VII. ANTHRAX	228
VIII. DIPHTHERIA	240
Diphtheria in England—The Diphtheria Bacillus—The Pseudo-Diphtheria Bacillus—Clinical Diagnosis—The Xerosis Bacillus—Diphtheritic Affections of Birds and Animals.	
IX. 'ACID-FAST' BACILLI—TUBERCULOSIS—LEPROSY—THE SMEGMA BACILLUS—GLANDERS	276
X. TYPHOID FEVER—PARA-TYPHOID FEVER—BACILLUS ENTERITIDIS AND THE GÄRTNER GROUP—SWINE FEVER—BACILLUS DYSENTERIÆ—BACILLUS COLI . . .	326

MANUAL OF BACTERIOLOGY

	PAGE
RUSSIAN PLAGUE—CHICKEN CHOLERA—MOUSE SEPTIC- ÆMIA	366
PNEUMONIA AND INFLUENZA	388
ANAEROBIC ORGANISMS—TETANUS—MALIGNANT ŒDEMA —BLACK QUARTER—BACILLUS WELCHII (AEROGENES CAPSULATUS, ENTERITIDIS SPOROGENES)—BACILLUS CADAVERICUS SPOROGENES—CLOSTRIDIUM BUTYRICUM .	395
ASIATIC CHOLERA—SPIRILLUM METCHNIKOWI—SPIRIL- LUM OF FINKLER AND PRICE—SPIRILLUM TYROGENUM —SPIRILLUM RUBRUM	408
STREPTOTHRIX INFECTIONS—ACTINOMYCOSIS—MYCE- TOMA—LEPTOTHRIX BUCCALIS—CLADOTHRIX DICHOTOMA— MYCOSIS TONSILLARIS	425
THE BLASTOMYCETES	437
Saccharomycetes and Torulae—The Common Yeasts and their Examination—The Pathogenic Blastomycetes.	
THE HYPHOMYCETES—RINGWORM AND THRUSH . . .	446
THE PROTOZOA	457
The General Structure of the Protozoa—Pathogenic Amœba—Trypanosomata—Leishman-Donovan Body— Spirochaete—Coccidia—Malaria.	

LIST OF PLATES

PLATE

I. PHAGOCYTOSIS AND <i>M. PYOGENES</i>	<i>to face p. 204</i>
II. <i>STREPTOCOCCUS PYOGENES</i>	" 210
III. THE MENINGOCOCCUS AND GONOCOCCUS	" 222
IV. ANTHRAX	" 228
V. ANTHRAX	" 230
VI. DIPHTHERIA	" 242
VII. THE HOFMANN BACILLUS AND VINCENT'S ANGINA	" 272
VIII. THE TUBERCLE BACILLUS	" 276
IX. LEPROSY AND <i>B. SMEGMATIS</i>	" 310
X. <i>B. MALLEI</i> AND GLANDERS NODULE	" 322
XI. <i>BACILLUS TYPHOSUS</i>	" 328
XII. <i>B. TYPHOSUS</i> AND <i>B. COLI</i>	" 356
XIII. PLAGUE	" 368
XIV. <i>B. PESTIS</i> AND CHICKEN CHOLERA	" 370
XV. <i>DIPLOCOCCUS PNEUMONIÆ</i>	" 386
XVI. <i>B. TETANI</i> AND <i>B. WELCHII</i>	" 398

MANUAL OF BACTERIOLOGY

<i>SPIRILLUM CHOLERÆ</i> AND CULTURES OF	
<i>SPIRILLA</i>	to face p. 410
<i>ACTINOMYCOSIS BOVIS</i> AND MYCETOMA	" 430
<i>ACTINOMYCOSIS HOMINIS</i>	" 433
<i>TRYPANOSOMA BRUCEI</i> AND <i>SPIROCHAETA</i>	
<i>RECURRENTIS</i> (OBERMEIERI)	" 436
<i>COCCIDIUM OVIFORME</i> AND <i>HALTERIDIUM</i>	
<i>DANILEWSEYI</i>	" 473
THE MALARIA PARASITE	" 486
TERTIAN 'ROSETTE' AND <i>PIROPLASMA</i>	
<i>CANIS</i>	" 496
<i>TREPONEMA PALLIDUM</i>	" 532

A MANUAL OF BACTERIOLOGY

INTRODUCTION.

Bacteriology is that branch of Biology which deals with the study of Micro-organisms, particularly the minute vegetable organisms known as *Bacteria*. The scope of bacteriology is difficult to define exactly, the term often being used in a comprehensive sense equivalent to micro-pathology, or even micro-biology, for all investigations connected with micro-organisms, animal and vegetable, may be included under it. So extensive, however, has the subject become that the animal micro-organisms are now being studied as a separate branch, Protozoology. Bacteriology deals with micro-organisms particularly in their relation to processes—disease, fermentation, putrefaction, and the like—while their structure, functions, and life-history are to a large extent left to the botanist and zoologist. There is no space in a work of this kind to enter into the history of the science, but the names of Leeuwenhoek (1675), Müller (1786), Schwann (1837), Cohn, Pasteur, Lister, and Koch will ever hold an honourable place in its annals.

The study of micro-organisms must always be of considerable importance in general biology, for their vital

phenomena are comparatively simple, and throw much light on the more complex processes occurring in the higher orders of living beings. Weismann based his theory of heredity on the fundamental conception of the immortality of these unicellular organisms. Excluding accidents, they are immortal—they reproduce themselves by a process of simple division, an individual dividing into two, and two daughter forms taking the place of the original parent one, and although the parent has disappeared yet there has been no death, no dissolution; its protoplasm or living material is still existent in its progeny and is immortal, since this process of reproduction apparently may go on indefinitely. Moreover, the study of the possibility of the transformation of species of micro-organisms is likely to throw light on the theory of evolution. Organisms such as bacteria multiply so rapidly that fifty or sixty generations may be developed in thirty hours, a number which would take years to attain if even the most rapid breeder among mammals were the subject of experiment, and as they occur in vast numbers there is a wide field for variation. These are some of the points of the relation of micro-organisms to general biology.

In what may be termed the economy of nature micro-organisms are all-important, without them there would be no putrefaction, no decay, and the dead remains of animal and vegetable life would so accumulate as to encumber the earth, which would become sterile for the want of the organic matter originally derived from it, but of which there was no return. In fact the higher plants, and indirectly, therefore, animals also, are dependent for their existence upon the presence of bacteria in the soil, which break up and render assimilable complex substances presented to them as manures.

The question of life, animal and vegetable, without bacterial activity is an important and interesting one. It

would seem from the experiments of Duclaux¹ that the higher plants in ordinary circumstances are unable to obtain nutriment unless the complex compounds, proteins, urea, and nitrogenous bodies, which form the important constituents of many manures, are broken down into simpler ones through the agency of bacteria. He sowed seeds in sterile soil free from nitrates, nitrites, and ammonia, which was plentifully watered with sterile milk and solutions of sugar and starch. No changes occurred in these substances, the seeds lost weight, and the seedlings dwindled and died. As regards the higher animals various views have been expressed. Pasteur considered that their life also would probably be impossible without the presence of bacteria in the intestinal tract. Nencki expressed the opinion that this idea of Pasteur's was an erroneous one, and his experiments in conjunction with Macfadyen and Sieber² showed that any considerable decomposition of the food by bacteria first takes place in the large intestine, and that the digestive juices alone, without the co-operation of bacteria, are able to prepare the constituents of the food for absorption. Nuttall and Thierfelder obtained unborn guinea-pigs by Cæsarian section with antiseptic precautions, and afterwards kept them in a sterile environment and fed them on sterilized food. Not only did the animals live, but they were even in a more thriving condition than those naturally brought up. The intestinal tract was found to be sterile on the eighth day. On the other hand, Schottelius found that chickens reared on sterile food were retarded in development, and experiments by Moro on turtle larvæ lead to the same conclusion, viz. that intestinal bacteria are necessary for normal nutrition. Levin, however, found that the intestinal tract in many Arctic animals, the polar bear, reindeer, seal, eider duck, &c., is generally sterile, and

¹ *Comp. Rend.* T. 100, p. 66.

² *Journ. of Anat. and Physiol.* xxv. p. 390.

in these instances, therefore, bacteria are not required for normal nutrition.

Commercially micro-organisms are of the utmost importance. Without them there would be no fermentation, and the wine, beer, and indigo industries, the ripening of cheese and tobacco, and many like processes would be non-existent. From a financial aspect also micro-organisms cannot be ignored, for many of the so-called 'diseases' of beer and wine, which often occasion great loss, are due to the entrance of adventitious forms, while the silk industry and sheep farming in France were once threatened with extinction owing to the ravages of pébrine and of anthrax respectively, but through the genius of Pasteur were restored to their former prosperity. There is no need to emphasize the importance of micro-organisms from a medical and hygienic point of view, but the fact may be recalled that fifty years ago the mortality after operations was very high, and that 40 per cent. of these deaths were caused by pyæmia, septicæmia, and hospital gangrene, conditions which are due to the entrance of micro-organisms, and which are now almost preventible, thanks to the anti-septic system introduced by Lord Lister.

The theory of spontaneous generation or abiogenesis is intimately connected with the study of bacteria. The putrefaction of animal and vegetable fluids even after boiling, and the growth in them of minute living forms, were held by many to be a sure proof of the development of life from inanimate matter, of the spontaneous generation of the living from the non-living. A succession of investigators, however, showed (1) that if the fluids were boiled sufficiently long, and then sealed up so as to prevent the access of air, they did not undergo putrefaction; (2) that the sealing up could be dispensed with, provided the air were first filtered through cotton-wool before being admitted to the flasks; (3) that even the cotton-wool was not needed if the air

were passed slowly through a long and tortuous channel, so as to deposit its solid particles. Tyndall showed that putrescible fluids might be exposed in open vessels in a closed chamber in which the air had been undisturbed for some time and its solid particles thereby deposited on the walls of the chamber, which had been smeared with glycerin; he also proved that vegetable infusions and the like, which putrefied after having been boiled for ten minutes, did not do so if the boiling were repeated on two or three successive days, and explained this by the supposition, that while the fully developed bacteria were destroyed by the first boiling, their more resistant spores remained alive, but these on being left for twenty-four hours germinated into the less resistant bacterial forms, which were destroyed by the second boiling, and by the repetition of the process complete sterilization was ultimately obtained. It is this process of 'discontinuous sterilization,' as it is termed, which is employed by the bacteriologist for the preparation of sterile culture media.¹

The idea of abiogenesis (or as he prefers to term it, archebiosis) has recently been revived by Bastian. He claims that certain vegetable infusions and saline solutions which have been boiled or even heated above the boiling-point in sealed tubes after a time show the development of various living organisms including bacteria and yeasts.²

Dunbar,³ as the result of a series of experiments conducted over a long period and with every care to prevent contamination, has come to the conclusion that the bacteria are not an independent group of organisms, but that the

¹ The writer believes that this explanation is only partially true, and would ascribe some of the sterilizing effect of repeated heatings simply to the injurious action of alternate heating and cooling.

² See various papers in the *Proc. Roy. Soc. Lond.* and *The Evolution of Life*. Methuen, 1907.

³ See *Journ. Roy. Inst. Pub. Health*, xv. No. 11, 1907, p. 679.

bacteria, yeasts and moulds are stages in the life-history of green algæ. The observations were carried out both by culture methods and by microscopical examination. A culture of a single-celled alga belonging to the *Palmellacea* was obtained, but by modifying the culture medium in which a pure culture of the alga was growing, by the addition of acid, of alkali, or of traces of copper salts, other organisms, generally bacteria, occasionally moulds and yeasts, and even spirochaetes, made their appearance. Granting that there is no flaw in the experimental methods, and every care seems to have been taken to exclude contamination, &c., the results are susceptible of another explanation, viz. that the secondary growths were derived by transformation of the algal cells, in fact by the phenomenon of heterogenesis which has been claimed by Bastian to occur.

Doubtless immense progress has been made during the last two or three decades, but a vast amount still remains to be done. We have only touched the fringe of the explanation of the difficult problems of immunity, of the extraordinary variations in virulence and effects of the same organism, and of the important question of cure in, and prevention of, infective diseases, while the chemistry of the products of bacterial activity is still in its infancy.

The literature of Bacteriology is now becoming somewhat extensive. In the following pages a good many references to original papers have been introduced, so that further information may be obtained if required, the aim being as far as possible to refer to easily accessible papers which contain a more or less full bibliography on a particular subject.

The English journals containing papers on bacteriology are the 'Journal of Pathology and Bacteriology,' 'The Lancet,' 'The British Medical Journal' and its useful Epitome, and the provincial medical journals; 'The Medico-Chirurgical' and 'The Pathological' Societies' Transactions (now merged in the

'Transactions' and 'Proceedings of the Royal Society of Medicine'), the 'Proceedings' and 'Transactions of the Royal Society of London,' the 'Journal of Hygiene,' the 'Journal of the Royal Microscopical Society' (abstracts), the 'Journal of the Royal Institute of Public Health' (hygienic), the 'Journal of Comparative Pathology and Therapeutics' (veterinary), and the 'Brewers' Journal' (fermentation &c.) In America the medical journals, the 'Journal of Experimental Medicine,' the 'Journal of Medical Research,' and the 'Journal of Infectious Diseases,' also contain valuable bacteriological papers. The French journals are the 'Annales de l'Institut Pasteur,' the 'Bulletin de l'Institut Pasteur' (abstracts), and various medical journals. In German, the 'Centralblatt für Bakteriologie und Parasitenkunde' (both parts), 'Zeitschrift für Hygiene,' 'Hygienische Rundschau,' and the medical journals generally. Baumgarten's annual 'Jahresberichte' (pathogenic organisms) and Koch's annual 'Jahresbericht' (fermentation &c.) give valuable epitomes, but the papers referred to are a year or two old.

A very full bibliography is given in Sternberg's 'Manual of Bacteriology' (up to 1893), in Flugge's 'Die Microorganismen,' and in Kolle and Wassermann's 'Handbuch der Pathogenen Mikroorganismen,' the most encyclopedic work on this branch of bacteriology yet published. The volumes of the 'Index Catalogue' of the Surgeon-General's Library at Washington may also be consulted. A valuable bibliography will be found appended to the various articles in Clifford Allbutt's 'System of Medicine.'

An index to current literature will be found in the volumes of the 'Index Medicus,' and to papers a year or so old in the 'International Catalogue of Scientific Literature.'

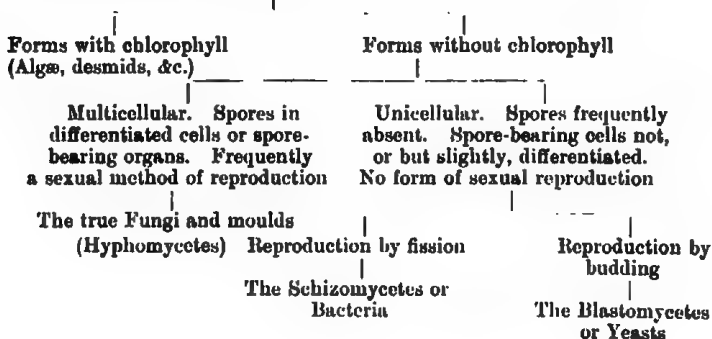
For the determination of species of bacteria Chester's 'Manual of Determinative Bacteriology' (Macmillan, 1901) will be found invaluable. He suggests a nomenclature for the description of cultural characters &c. which it would be very convenient to adopt. It includes a Bibliography of all the forms described. The 'Atlas' of Lehmann and Neumann (Saunders) is also very useful.

CHAPTER I.

THE NATURE, STRUCTURE, AND FUNCTIONS OF THE BACTERIA :
 THEIR CLASSIFICATION, GENERAL BIOLOGY, AND CHEMISTRY
 —BACTERIA AND DISEASE.

The Bacteria or Schizomycetes ('fission fungi') are minute vegetable organisms for the most part unicellular and devoid of chlorophyll, which multiply by simple transverse division or fission; this distinguishes them from the yeasts, in which multiplication takes place by budding or gemmation. A certain number of filamentous forms are also included, serving to connect the unicellular ones with the multicellular true fungi. Their relation to the other lower plants is shown in the following scheme :

Thallophyta (lower plants without fibro-vascular bundles, and with no distinction between root and stem)



The unicellular plants are sometimes termed the Proto-phyta. It must be understood that there are connecting links between the different groups, and that there is no sharp line of demarcation between them.

The size of the Bacteria is variable, but they are all microscopic, measuring from 0.3μ to $30-40\mu$ in diameter or in length. Their shape likewise is very different in the different species; some are spherical, others ovoid, others rod-shaped or filamentous, while in some the rod or filament is twisted into a spiral. The bacterial cell consists of a cell-membrane enclosing the transparent, more or less structureless living matter or protoplasm, the cell-plasma or cytoplasm. The cell-membrane in a few species may be composed of cellulose, but in the majority it consists of a protein substance. Bütschli has described the bacterial plasma as having a reticular structure, but in the young cell this is probably either an artifact or a 'false image' due to faulty illumination; the most that can be seen is a fine granulation. The protoplasm frequently contains larger granules composed of fatty or protein matter, pigment, and in some species of sulphur. Occasionally certain granules stain blue with iodine. One to three spherical granules are also present in each cell; these seem to take part in the division of the cell (see below), and if the latter be stained with a very dilute mixture of roseine and methylene blue are coloured pink, contrasting with the rest of the protoplasm, which is stained blue.

Unless the roseine-staining granules, just mentioned, be regarded as such, no nuclear structure is present in the cell, nor does the theory which regards the bacteria as primitive nuclei almost devoid of protoplasm seem to be tenable. The protoplasm when dead, according to Nencki, differs from ordinary protein matter in not being precipitated by alcohol and in not containing sulphur; it was

termed by him 'myko-protein.' This does not appear to be the case with the proteins obtained by grinding bacterial cells, which seem to agree with other proteins in heat-coagulation &c.

The cell-membrane is usually invisible, but if the cell is treated with salt-solution (2·5 per cent.) *plasmolysis* takes place, the protoplasm shrinking away from the membrane, which then becomes visible. It can also be stained *in vivo* with very dilute solutions of roseine. The cell-membrane sometimes becomes thickened, swollen, and gelatinous on its outer surface, forming a layer or so-called 'capsule' around the organism. When large groups of bacteria become thus encapsuled, they adhere together in a sort of jelly-like matrix, forming what is known as a 'zoogloea.' The clear spaces frequently seen around bacteria in dried and stained preparations, especially in those from blood and lymph, are generally artifacts and not true capsules. In *Cladothrix* and some other forms the cell-membrane becomes hardened, leading to the production of a firm sheath.

All species of bacteria, but especially the smaller ones, when suspended in a fluid exhibit what is known as Brownian movement, consisting of an oscillation with some amount of rotation about a fixed point, but there is little actual movement of translation, unless due to flotation. This Brownian movement is physical and not vital in nature, and occurs with all fine particles suspended in a fluid, and must be clearly distinguished from a true vital motility. Some bacteria are always motionless, others are more or less motile, but these, too, have a resting stage. For motility to occur the cells must be young, and the conditions favourable to growth and development. Motility seems to be due to delicate protoplasmic threads termed flagella connected with the outer layer of the cell protoplasm; these vibrate to and fro and propel the organism

through the medium. A cell will, however, move indifferently in either direction; if a motile organism be watched it will often be seen to proceed rapidly in one direction, stop, and then return without turning round. The flagella are not visible in the living state, unless dark ground illumination with a paraboloid be used, nor by the ordinary methods of staining, unless previously treated with a mordant, and are extremely liable to be broken off. They vary considerably in number and in length; some organisms have but a single flagellum at one pole (*monotrichic*), e.g. *Bacillus pyocyaneus*, others have two or more flagella forming a brush or tuft (*lophotrichic*), e.g. *Spirillum rubrum*, while others may be almost entirely covered with them (*peritrichic*), e.g. *B. typhosus*; in some the flagella are short and straight, and in others long and twisted. The motility of organisms does not necessarily depend directly upon the number of flagella they possess, an organism with a few flagella being often more active than another possessing many, and some are apparently non-motile, though well-marked flagella can be demonstrated. Generally speaking, however, an organism with several flagella will be more motile than a similar form with a few. (See also Chap. IV.)

Darwin says, 'In looking at nature it is most necessary never to forget that every single organic being may be said to be striving to the utmost to increase in numbers,' and in no group perhaps of the animal and vegetable kingdoms is this more marked than among the bacteria. Reproduction is entirely non-sexual, and takes place in two ways—by simple division or fission and by spore formation. Reproduction by transverse fission is common to all bacteria; the bacterial cell becomes constricted at its middle and finally separates into two parts, and thus two young cells take the place of the parent one; reproduction by fission is therefore also an increase in numbers. The

fission is always transverse, never longitudinal,¹ the rule being in cell-division that the new membrane is formed in the most economical manner. Longitudinal division, on the other hand, seems to be very common among the Protozoa. Previous to division the rod forms become elongated and the spherical ones ellipsoidal, and there is an increase in the number of the roseine-staining granules, partly by division of pre-existing ones and partly by new formation. The constriction in the majority of cases involves and passes through one of the granules. In the monotrichous and lophotrichous bacteria it is always the non-flagellated end of the dividing cell which bears the flagella of the new cell. Under favourable conditions reproduction may be very rapid, fission occurring every twenty or thirty minutes (Klein), so that, the increase being in a geometrical ratio, the number of individuals which might arise from a single bacterium in three or four days is almost inconceivable, and would *en masse* weigh thousands of tons; fortunately, there are many checks to such a rapid multiplication. Frequently, although the protoplasm divides, the division of the cell-membrane is incomplete, resulting in a loose union of the cells with the formation of a pseudo-filament. These filaments often become much curved and twisted, forming tangled masses, owing to fission taking place in the cells in the middle of the filament as well as at the ends, so that the filaments have to become curved to make room for the new cells.

Reproduction by spore formation is met with in some species, and is generally described as being of two kinds. In the first, 'endogenous' spore formation, a bright refractile round or ovoid body is formed within the bacterial cell, the development of which can be watched under the micro-

¹ Longitudinal division has been described in a few species, but its occurrence is so rare that a doubt must arise as to whether these forms are true bacteria.

scope. Rowland describes the process of spore formation as follows: refractile, oily-looking droplets, which do not stain with roseine, appear and ultimately coalesce, forming the spore. The cell-plasma at the same time diminishes and retracts from the cell-membrane. The roseine-staining granules increase in number and aggregate into two spherical masses, which dispose themselves one at each end of the cell. The cell-membrane collapses somewhat, and, when the spore is fully formed, ruptures transversely, leaving two cup-shaped receptacles, in which the granules and remains of the plasma are still recognizable. Spores are single, one only forming in each cell, and they seem to fulfil the purpose of perpetuating the race when it is threatened with extinction from adverse circumstances. Each consists of a little mass of protoplasm enclosed within a very tough and resisting membrane, which preserves its vitality even under unfavourable conditions; for spores resist the action of desiccation, heat, and germicidal agents to a much greater degree than the fully developed organisms. Spores vary much in size and in the position they occupy within the bacterial cell in the different species; their diameter is usually about the same as that of the cell in which they are developed, but may be much greater, and in position they may be central or terminal, and sometimes the spore-bearing cells are swollen or club-shaped; these are termed 'clostridia.' Endospores are still unknown in a large number of species. The second variety of spore formation, 'arthrospore' formation, is of doubtful occurrence. Some of the elements formed by fission are slightly larger, more refractile, and more resisting than their fellows, and are stated to have the properties of spores. Placed in favourable circumstances, the spore in either case germinates, it becomes swollen and granular, and loses its refractile appearance; a slight protuberance forms, this increases in size, and an organism similar to the parent

one is finally reproduced, the empty spore membrane at first frequently enclosing one extremity, and then being cast off. In certain instances the spore germinates without casting its membrane, the spore membrane becoming the cell-wall of the young organism. The ellipsoidal spores of the *B. anthracis* sprout at the end, those of *B. subtilis* at the side.

On the Morphology and Reproduction of the Bacteria see Rowland, *Trans. Jenner Inst. Prev. Med.* ii., 1899, p. 148; Fischer, *The Structure and Functions of Bacteria*, 1900; Migula, *System der Bakterien*, i.

CLASSIFICATION OF THE BACTERIA.

Many classifications of the bacteria have been proposed, but none up to the present can be said to be strictly scientific, or even satisfactory from the point of view of convenience. In the first place, the bacteria are said to be devoid of chlorophyll, but there are many forms intermediate between those unicellular organisms with and those without chlorophyll, so that a hard and fast line cannot be drawn. In the next place, the bacterial cells are so minute, and their vital phenomena so simple, that only a few broad distinctions can be observed in their morphology and reproductive processes.

One of the most prominent of the older classifications was that of Cohn. He divided the bacteria into four main groups:

- I. The Sphærobacteria or spherical forms.
- II. The Microbacteria or short rod-forms.
- III. The Desmobacteria or long rod-forms.
- IV. The Spirobacteria or spiral forms.

Zopf's classification (1885) has many points to commend it, but is largely based on the doctrine of pleomorphism

By pleomorphism is meant a variation in the form of an organism during its life-cycle, a coccus, for example, growing into a rod, or a straight rod becoming a spiral. In a peach-coloured bacterium examined by Lankester, cocci, rod, filamentous and spiral forms occurred, and the doctrine of pleomorphism received considerable support from his work; but some doubt has been cast on it, and it is suggested that he was not working with pure cultures. Be that as it may, a certain amount of pleomorphism undoubtedly occurs in some organisms. In the colon, typhoid, and plague bacilli, for example, the rods may sometimes be so short as to be almost cocci, while at others they are well-marked rods and even filaments. The following is an outline of Zopf's classification, the bacteria being divided into four main groups or families, which again are subdivided into smaller groups or genera:

Family I. COCCACEÆ.—Spherical forms only; division occurs in one or more directions.

Genus 1. MICROCOCCUS (Staphylococcus).—Division in one direction only, but irregular, so that the cocci after division form irregular clusters.

Genus 2. STREPTOCOCCUS.—Division in one plane, but regular, so that the cocci form chains.

Genus 3. MERISMOPEDIA.—Division in two directions at right angles to each other, but in the same plane, so that lamellæ or plates are formed.

Genus 4. SARCINA.—Division in three directions at right angles to each other and in two planes, so that cubical masses are formed.

Genus 5. ASCOCOCCUS.—Cocci which develop in a gelatinous matrix.

Family II. BACTERIACEÆ.—Rods, straight or curved, at some period of the life-history, though cocci and other forms may occur.

Genus 1. BACTERIUM.—Straight rods, endospore formation does not occur.

Genus 2. BACILLUS.—Straight rods, endospore formation occurs.

Genus 3. LEUCONOSTOC.—Cocci and rods, arthrospore formation occurs in the cocci forms.

Genus 4. CLOSTRIDIUM.—The same as bacillus, but the spore-bearing rods are enlarged, and club-shaped.

Genus 5. SPIRILLUM.—Spiral rods, spore formation does not occur.

Genus 6. VIBRIO.—Spiral rods, spore formation occurs.

Family III. LEPTOTRICHEÆ.—These are unbranching thread forms.

Family IV. CLADOTRICHEÆ.—These are thread forms showing true but not dichotomous branching.

There are many points in this classification which are of practical value. The distinction made between a bacterium and a bacillus, for example, is very convenient. Formerly it was the custom to term a short rod a bacterium, and a long rod a bacillus, but such a division is an arbitrary one, and at one stage of its life-history an organism might have to be termed a bacterium and at another a bacillus. The term 'bacterium' is now but little used, and any straight rod is termed a bacillus. The term 'staphylococcus' is one frequently met with; it is practically synonymous with micrococcus, and refers to cocci which are aggregated into groups or clusters. Of the twisted rods, a simple curved rod is now known as a vibrio, a definitely corkscrew form of three or a few turns is a spirillum, a long and flexible twisted filament is a spirochæta.¹

¹ Many of the so-called spirochæte are probably protozoa and not bacteria.

The latest system of classification is that proposed by Migula.¹ The bacteria are divided into two orders: the Eubacteria, bacteria proper, the cells of which contain neither sulphur granules nor a colouring matter, bacteriopurpurin; and the Thiobacteria, the cells of which contain sulphur granules and may be coloured with bacteriopurpurin. The Eubacteria are divided into five families: (1) *Coccaceæ*, (2) *Bacteriaceæ*, (3) *Spirillaceæ*, (4) *Chlamydo-bacteriaceæ*, and (5) *Beggiatoaceæ*. These, again, are subdivided into many genera, based partly on the mode of division and partly on the number of flagella and their arrangement on the organisms. The *Coccaceæ* contain the genera *Streptococcus*, *Micrococcus*, *Sarcina*, *Planococcus*, and *Planosarcina*, the *Bacteriaceæ* are defined as long or short cylindrical rods, straight and never spiral; division in one direction only after elongation of the rods; and this family has three genera: (a) *Bacterium*, non-flagellated cells, often with endospore formation; (b) *Bacillus*, cells possessing both lateral and polar flagella, often with endospore formation; (c) *Pseudomonas*, cells with polar flagella only, rarely endospore formation.

The nomenclature of bacterial species is at present in a chaotic condition. In botanical and zoological nomenclature every species has a binomial name, the first being the generic, the second the specific, name. Many bacterial species have received trinomial names, which should be inadmissible. The specific name first given to an organism must stand unless it has been used for some other species.

CONDITIONS OF LIFE OF BACTERIA.

Bacteria, being living organisms, must be supplied with suitable nutritive substances in order that their life-processes, nutrition, reproduction, and the like, may be carried

¹ *System der Bakterien*, 1897. Abstract in *Centr. f. Bakt.* (1^{re} Abt.) xxii., 1897 (September), p. 345.

on in a normal manner. Being devoid of chlorophyll they are mainly dependent upon complex organic compounds for the carbon, hydrogen, and nitrogen which enter into their composition, these elements being derived for the most part from proteins and carbohydrates. Some bacteria, however, are able to obtain the requisite nitrogen from such comparatively simple compounds as ammonia, ammonium carbonate, or nitrates, and one group can make direct use of the atmospheric nitrogen. Certain inorganic salts, sulphates, phosphates, and sodium chloride, also seem to be necessary for normal development. These nutrient substances must be presented to the bacteria in association with water, for without water bacterial activity ceases, though in the dry state many forms, and especially their spores, may retain their vitality for a considerable time; absolute desiccation, however, is probably rapidly fatal.

Temperature is also an important factor. Though the growth of many species occurs through a wide range, there is for almost all an optimum at which growth is best, and of a range not exceeding 5 or 10 degrees. Growth usually ceases below 10° C., but cold does not destroy bacterial life; after exposure to the intense cold produced by the evaporation of liquid oxygen (— 170° C.) for weeks, or of liquid hydrogen (— 252° C.) for ten hours, bacteria and their spores will grow and germinate, and their chromogenic and pathogenic properties seem to be unaltered.¹ On the other hand, bacterial growth usually ceases when the temperature exceeds 40° C. or thereabouts, and most bacteria without spores are destroyed in half an hour or so by a temperature of 65° C. The spores are far more resistant, some may even be boiled for a short time without losing their vitality, but prolonged boiling is fatal to both bacteria and their spores. There is, however, a group of so-called thermophilic bacteria, which

¹ Macfadyen and Rowland, *Proc. Roy. Soc. Lond.* Feb. 1, 1900; April 5, 1900; May 31, 1900.

thrive best at a temperature of 60° to 70° C. They occur in the soil and in water, and are probably of considerable importance in the natural fermentations accompanied by the evolution of heat, such as are met with in manure heaps, the heating of hay, and firing of moist cotton.¹

Free oxygen is essential to the growth of some organisms; these are termed strictly aerobic. Others will not develop in its presence, strictly anaerobic; others again, while preferably aerobic or anaerobic, will grow in the absence, or in the presence, of oxygen, and are respectively termed facultative anaerobic or facultative aerobic. Some organisms are strictly parasitic on animals or plants; others live in water, soil, decaying matter, &c., these are termed saprophytes; and many are able to exist either as parasites or as saprophytes (see also p. 134).

Bacterial development is much influenced by the presence of foreign substances in the nutrient medium. A number of bodies, metallic salts, chlorine, bromine, and iodine, carbolic acid, salicylic acid, &c., have an injurious effect upon bacterial life, inhibiting or stopping growth, or killing the organisms outright; these are of considerable practical importance and are known as germicides, antiseptics, and disinfectants. The products produced in the nutrient medium by the bacteria themselves also sooner or later inhibit or stop further growth; a familiar instance of this is seen in the alcoholic fermentation of sugar by yeast, which ceases when the amount of alcohol reaches 12 or 14 per cent. The same reason probably explains why the growths of bacteria in culture tubes do not spread all over the surface of the nutrient medium, and why our cultures sometimes die out more rapidly than might be expected.

Another point affecting bacterial life is the presence of a mixture of organisms in the same nutrient medium.

¹ Macfadyen and Blaxall, *Journ. of Path. and Bact.* Nov. 1894, and *Trans. Jenner Inst. of Prev. Med.* ii., 1899, p. 162.

If there be a very vigorous form, it may ultimately grow and multiply to such an extent as to crowd out and finally kill the other forms with which it is associated, and if the nutrient medium equally favour two species, that one which is in an excess at the beginning may crowd out the other. The occurrence of what has been termed symbiosis is of considerable interest in the life of micro-organisms, and too little attention has hitherto been paid to it. This is the coexistence of two or more species which together bring about certain changes. For example, in the well-known ginger-beer plant, Marshall Ward¹ isolated several yeasts, bacteria, and moulds; of these, one of the yeasts and one of the bacteria together induce the particular changes in a saccharine fluid to which ginger has been added, which render the mixture like ginger-beer, and these changes do not occur unless both species develop together.

Another extraordinary feature exhibited by bacteria is the selective action exerted on certain substances which contain isomerides or right- and left-handed modifications of a substance. The *Bacillus ethaceticus* attacks mannitol but not dulcitol, two alcohols which are very similar in taste and properties and possess the same chemical formula.

By a series of most brilliant researches Emil Fischer has succeeded in determining the constitution of the various sugars, and, what is more, has produced them artificially in the laboratory. The natural sugars are all compounds with dissymmetric molecules, powerfully affecting the beam of polarised light, but when prepared artificially they are without action on polarised light, because the artificial product consists of equal numbers of left-handed and right-handed molecules, and the molecules of the one neutralize the molecules of the other, thus giving rise to a mixture which does not affect the polarized beam.

¹ *Phil. Trans. Roy. Soc. Lond.* 1892, vol. 183, p. 125.

By the action of micro-organisms, however, on such an inactive mixture the one set of molecules is sought out by the microbes and decomposed, leaving the other set of molecules untouched, and the latter now exhibit their specific action on polarized light, an active sugar being thus obtained.

Fructose was one of the principal artificial sugars prepared by Fischer; it is inactive, but consists of an equal number of molecules of oppositely active sugars termed 'levulose.' One set of these levulose molecules turns the plane of polarization to the right, another set to the left, right- and left-handed levulose. The left-handed levulose occurs in nature, while the right-handed levulose, so far as is known, does not.

Now, on putting brewer's yeast into a solution of fructose, the inactive artificial product, the yeast organisms attack the left-handed levulose molecules and convert them into alcohol and CO_2 , while the right-handed levulose is left untouched.

Pressure, unless very great, has little effect on bacteria. Roger investigated the effects of high pressure on certain organisms in bouillon cultures. Pressures of 200 to 250 kilos. per square centimetre had no effect; by raising the pressure to 3000 kilos. per square centimetre one-third of streptococci were killed, and of anthrax without spores a good many; while sporing anthrax, *Micrococcus pyogenes*, var. *aureus*, and the colon bacillus were unaffected.¹

Our countrymen Downes and Blunt first called attention to the injurious effect of light upon bacteria. If plate cultures be prepared and exposed to sunlight, a portion of the plate being protected from its action, as by sticking on a letter cut

¹ Bacteria being so minute, the actual pressure on a bacterial cell, even with these high pressures, is small. If, for example, a bacterium measures 1μ by 5μ , a pressure of 1000 kilograms per square centimetre would be but 0.05 gram ($\frac{1}{2}$ grain) on the cell.

out of black paper, and the preparation afterwards incubated, it will be found that the colonies develop at the protected portion only, those parts which have been exposed to sunlight remaining sterile. Although this action of sunlight may occasionally be due to chemical changes in the medium, resulting in the production of ozone or other germicidal bodies, the experiments of Marshall Ward and others have conclusively shown that germicidal action may be caused by the direct action of the light, the violet and ultra violet rays being those concerned, and the red end of the spectrum has no effect. The Röntgen rays seem to have little or no influence upon bacteria, but the results obtained are somewhat contradictory.

The radium emanations with prolonged exposure and near contact are germicidal to non-sporing organisms.¹

Electricity, *per se*, has also usually little effect. When the current is passed directly through the cultures electrolysis takes place, and the products formed may destroy the bacteria; currents of high potential, however, may inhibit growth.²

Living motile bacilli are very sensible to induced currents of electricity, immediately orientating themselves in the direction of the current, while dead or paralyzed bacilli are unaffected.³

BACTERIAL PRODUCTS.

The chemical changes produced by micro-organisms are chiefly analytic or destructive, the formation of simpler from more complex bodies. This analytic faculty is present to a marked degree in what is known as putrefaction. *Putrefaction* is a term applied to the decomposition of organic, especially protein, matter after the death of the

¹ See Green, *Proc. Roy. Soc. Lond.* vol. 73, 1904, p. 375.

² LORTET, *Comp. Rend.* T. 119, 1894, p. 463.

³ *Comp. Rend.* T. 122, 1896, p. 892.

animal or plant. It is usually accompanied by the evolution of foul-smelling gases and by the solution of the solid material. A large number of organisms are concerned in this process, particularly a group to which Hauser gave the name of *Proteus*. The first changes which occur are the formation of proteoses and peptone, then leucin, tyrosin, and glyecol, and basic compounds to which the name of ptomine has been given, next indole, skatole, and phenol, and volatile fatty acids, and lastly mercaptans, sulphuretted hydrogen, marsh gas, ammonia, carbonic acid, and hydrogen.

In view of its practical importance in bacteriological analysis and the identification of species, indole may here be referred to at some length.

Indole.—Indole (C_8H_7N) is a product of the putrefactive decomposition of proteins containing a tryptophan nucleus and is formed during the growth of many organisms, and, since one species may produce it and another allied one may not, its presence or absence may be a valuable point in the identification of an organism. The detection of indole is based on the reaction with nitrous acid, with which it gives a fine purplish-red coloration. In order to test for it, the organism is grown in a fluid medium for twenty-four to forty-eight hours or longer, 1 c.c. of a 0.1 per cent. solution of sodium nitrite is added to every 10 c.c. of the culture, and a few drops of pure concentrated sulphuric acid or of hydrochloric acid are allowed to trickle slowly down the side of the test-tube, which is inclined with its mouth away from the operator. As the acid runs down, it is mixed with the fluid; a colour varying from pale pink to pale purple indicates the presence of indole. A control tube, uninoculated, should also be similarly tested to make sure that the reaction is due to the products of the growth of the organism. The culture fluid usually employed is peptone water, preferably 2 per cent., but some samples of 'peptone' occasionally fail to give the indole reaction when

MANUAL OF BACTERIOLOGY

ns are grown in media prepared from them; the
d of peptone must, therefore, be used. As the dilute
of sodium nitrite is unstable, a stock 5 per cent.
may be kept; 2 c.c. of this solution are diluted to
with distilled water at the time of making the test,
c. of this dilution is added to every 10 c.c. of the
The addition of the acid liberates free nitrous
which reacts with any indole present, and yields a
our. Sometimes when the reaction is apparently
r feeble, it may be obtained or intensified by placing
e in the blood-heat incubator for half an hour.
phuric acid must be pure and free from oxides of
, hence hydrochloric acid is often preferable.
ore delicate method of testing is to run a little
loric acid down the side of the tube, so that a layer
t the bottom, the nitrite having been previously
o the culture if required. A pink ring at the junc-
the hydrochloric acid and culture indicates the

The presence of dextrose, saccharose, glycerin, or lactose in quantity exceeding about 0.25 per cent. prevents the formation of indole in broth by bacteria. Broth prepared in the ordinary way usually contains a little dextrose derived from the glycogen in the meat, and this probably explains why the indole reaction is generally much more marked in a peptone water than in a broth culture, although the latter is a better nutrient soil. In order to prepare a soil free from dextrose, T. Smith¹ recommends that the acid beef broth used in the preparation of nutrient broth should be inoculated with the colon bacillus and incubated for twenty-four hours, and the peptone beef broth prepared from it. The dextrose is consumed and no indole is formed.

Some bacteria not only form indole but also produce nitrites in the culture medium by the reduction of the nitrates present in the peptone &c. used in making the nutrient medium, in which case the addition of pure sulphuric or hydrochloric acid alone suffices to bring out the pink indole reaction. This forms, therefore, an additional means of distinguishing organisms, and is employed especially for the recognition of the cholera spirillum, which, if grown in peptone water, gives the indole reaction (or, as it has been termed, 'the cholera red reaction') on the addition of acid alone. The reaction can be obtained as early as twelve hours after inoculation, and becomes very marked in twenty-four to forty-eight hours.

If indole is formed only in small quantities, 100 c.c. of the culture may be distilled; the first 20 c.c. of the distillate will contain the bulk of the indole.

This 'indole-reaction' is not necessarily always due to indole; the writer has shown² that the indole-like reaction obtained with cultures of the diphtheria and pseudo-diphtheria bacilli is owing to the presence of skatole-carboxylic

¹ *Journ. of Exper. Med.* ii., 1897, p. 543.

² *Trans. Path. Soc. Lond.* lii. pt. 2, 1901, p. 113.

MANUAL OF BACTERIOLOGY

This substance is distinguished from indole by being volatile. To make sure of the presence of indole, the should therefore be made alkaline with caustic soda and filtered.

Indole (methyl indole) seems also to be formed by some bacteria. It is volatile like indole, but if a solution containing it is boiled with an acid solution of dimethylamidobenzene (5 per cent. in 10 per cent. sulphuric acid) it yields a blue color, which gives to chloroform also a blue.

Nitrification.—Another important series of changes is included under the term 'nitrification.' As mentioned above, protein, albuminoid, and other complex nitrogenous compounds and urea, all of which are valuable manures for plants, cease to be so unless bacteria are present.

Through the agency of the putrefactive bacteria ammoniacal compounds are ultimately produced from the more complex nitrogenous substances. In order, however, that plants may obtain their requisite nitrogen, the nitrogen

filled with a mixture of 5 kilos. of ignited quartz sand and 100 grams of limestone. To this sewage was supplied at such a rate that it took eight days to pass through. During the first twenty days the sewage passed through unaltered; after that nitric acid began to appear in the filtrate, and increased to such an extent that the filtered sewage contained no ammonia but nitrates only. After passing sewage for four months with complete oxidation of ammonia, the authors placed a vessel of chloroform so that the vapour poured into the tube. In ten days after the introduction of the chloroform all nitrates disappeared in the exit water, and the sewage passed through unchanged.

After fifteen days the vessel containing the chloroform was removed. Nitrification did not resume, and after seven weeks the washings from ten grams of garden soil were added. Eight days after this addition nitrates again appeared in the exit water (this was confirmed by Warington).

Shortly after this Schloesing and Müntz found that exposure of soil to 100° C. for an hour destroyed the power of nitrification. Soils thus treated were exposed to a current of air, purified by ignition, without nitrification taking place; the addition of a little unheated mould was sufficient to cause nitrification to recommence. They also tried seeding the sterile soils with various *Hyphomycetes* &c. without result.

In 1884 Warington concluded that the factor determining the formation sometimes of nitric acid and sometimes of nitrous acid was a difference in the character of the organisms; for it is possible to have two similar solutions under identical conditions, and for nitrites to be produced in the one, and nitrates in the other.

In 1886 Dr. Munro showed that the process of nitrification could take place in solutions practically destitute of organic matter.

The methods of isolation and the nature of the organisms of nitrification must next be considered. Nitrification in the soil takes place in three stages :

I. *Ammonisation*.—When complex organic compounds such as albuminoids are applied to the land they are broken up ; first they become liquefied, peptone-like bodies being produced ; these are then further acted upon and we get alkaloidal substances in small quantity, indole, skatole, leucin, and tyrosin, valerianic acid, volatile fatty acids, lactic acid, &c.

These changes are brought about by numbers of organisms, among which the varieties of *Proteus* (formerly known as *Bacterium termo*) are the more important.

Then the nitrogenous compounds are further acted upon and ammonium salts formed. According to Emile Marchal, ammonization takes place essentially under the influence of microbes living in the upper layers of the soil. The *Bacillus mycoides* is one of the most energetic of these, and seems to play a double rôle, being ammonising both in the presence of nitrogenous organic substances and of nitrates. Urea is ammonized especially by the *Micrococcus ureæ*.

II. *Nitrosation*.—The ammoniacal salts are next converted into nitrites. The nitrous organisms can probably attack nitrogenous organic substances such as asparagine and milk, but only feebly, milk being much more rapidly nitrified when the nitrous organisms are mixed with other species.

III. *Nitratation*.—These nitrites are then converted into nitrates.

There is accumulated evidence to show that stages II. and III. are brought about by different species, the nitric organisms having no effect whatever on ammonia, but acting only after this has been oxidized into nitrous acid.

The discovery of Dr. Munro that organisms would grow in purely inorganic solutions has been made use of for the isolation of the different species. Solutions such as the following have been used :

For the Nitrous Organisms

Ammonium chloride 0.5 gm.
Potassium phosphate 0.1 gm.
Magnesium sulphate 0.02 gm.
Calcium chloride 0.01 gm.
Calcium carbonate 5.0 gm.
Distilled water 1000 c.c.

For the Nitric Organisms

Potassium nitrite 0.3 gm.
Potassium phosphate 0.1 gm.
Magnesium sulphate 0.05 gm.
Calcium carbonate 5.0 gm.
Distilled water 1000 c.c.

These are seeded with traces of earth, and by carrying on the cultivation for many generations a large number of organisms are eliminated. This method does not lead to a pure cultivation, for several forms besides the nitrifying organisms persistently maintain themselves in these mineral solutions.

So recourse was had to gelatin plate cultivations. Although several organisms were isolated in this manner, none of them possessed the slightest nitrifying power.

Frankland, and later Warington (1890), succeeded in isolating nitrous organisms by the dilution method. Nitrifying solutions were diluted, and traces inoculated into ammoniacal solutions; in some of these nitrification occurred, although no growth could be obtained on gelatin, and they were found to contain the nitrous organism only. A little later Winogradsky isolated nitrous organisms, first by modified gelatin plates, and afterwards by the silica jelly method.

Warington gives the following directions for the preparation of silica jelly plates: Sodium carbonate is fused in the blowpipe, and fine white sand is added as long as effervescence is produced. The mass is allowed to cool, and is then dissolved in water. The solution is poured into an excess of very dilute hydrochloric acid (silicic acid and sodium chloride being formed). The solution is

dialyzed and sterilized. Some of this is placed in a sterile dish and mixed with the following solution and inoculated :

Ammonium sulphate	0.4 gram
Magnesium sulphate	0.5 gram
Di-potassium hydrogen phosphate .	0.1 gram
Calcium chloride	trace
Sodium carbonate	0.6-0.9 gram
Water	100 c.c.

This mixture sets to a jelly in five to fifteen minutes.

Winogradsky has also made use of agar for plates, but this medium is not so suitable as the silica jelly. A 2 per cent. aqueous agar is prepared and poured into Petri dishes ; the film is then sown with *Proteus*, and allowed to grow for seven to ten days. It is then thoroughly washed, collected, melted, and mixed with the salts mentioned above. The object of growing the *Proteus* upon it as a preliminary is to get rid of the organic matter admixed with the agar.

The organisms isolated by Warington, Frankland, and Winogradsky were all nitrous forms.

Warington and Winogradsky in subsequent attempts succeeded in isolating nitric organisms. They occur in much the same form as the nitrous, viz. as round or ovoid corpuscles. These organisms, while having an energetic action in converting nitrites into nitrates, are without action on ammonia—in fact, this is inhibitory.

The nitrification of the soil is thus brought about by two groups of organisms. The first oxidizes ammonia into nitrous acid, and is isolated by successive cultivation in solutions of ammonium carbonate. The second group oxidises nitrous acid into nitric acid, and may be separated by successive cultivations in a solution of potassium nitrite containing a little sodium bicarbonate. In the soil the nitric and nitrous organisms are equally active.

The Leguminosæ, however, are able to obtain their nitrogen directly from the nitrogen of the air, also through

the agency of micro-organisms. If the roots of a pea, bean, or vetch be examined, numerous little nodules will be found upon them; on examining these microscopically small irregular bodies are found to be present, which have been termed bacteroids, and seem to be of the nature of involution forms. On inoculation into culture media the bacteroids give rise to a growth of organisms resembling bacteria; these 'fix' the atmospheric nitrogen. Leguminous plants grown from sterile seeds in a sterile soil dwindle and die, but if inoculated with the organisms derived from another plant of the same species growth becomes vigorous; if inoculated with those derived from another species growth still takes place, but not nearly to the same extent. The Leguminosæ thus store up one of the most important elements of plant food, and hence their value in the rotation of crops. A substance, termed 'nitragin,' consisting of a culture of these root organisms, has been prepared as a fertiliser. Unfortunately Nobbe's 'nitragin' did not prove a success. The work was continued by the United States Department of Agriculture and successful methods were eventually evolved, but the cultures tended to die in a few weeks. Further progress has been made by Professor Bottomley, who has succeeded in obtaining a powder preparation of the nitrogen fixing bacteria, which retain their vitality for months under these conditions, and the preparation properly applied to *poor* soils produces astonishing results.

Fermentation.—Another important group of changes produced by micro-organisms is that comprized under the comprehensive title of 'fermentation,' of which it is difficult to give an accurate definition, for the distinction between it and other chemical changes due to the activity of micro-organisms is conventional rather than scientific. Fermentation is brought about by the action of ferments, two classes of which are recognized, viz. the living or organized

ferments, which, in other words, are micro-organisms ; and the unorganized or chemical ferments, bodies such as pepsin, which in infinitesimal amount produce changes in a considerable quantity of the substance acted upon, without themselves undergoing alteration.

It is better to reserve the term 'fermentation' for the changes brought about by the organized ferments or living organisms, and to call the unorganized ferments enzymes, and the changes which they produce zymolysis. As fermentations are investigated more critically, the tendency is to find that they are brought about by enzymes, extra-cellular or intra-cellular, so that in course of time this distinction may no longer hold good. Many enzymes are secreted by micro-organisms—for example, the liquefaction of gelatin is due to an enzyme, and if a little of the liquefied gelatin free from organisms be added to fresh solid gelatin it will produce liquefaction of this, and the same occurs in the presence of chloroform, which inhibits bacterial action. Yeast also secretes enzymes ; it is unable to directly ferment cane sugar with the production of alcohol, but by the action of the enzyme 'invertase' the cane sugar becomes inverted, i.e. the molecule is split up into dextrose and lævulose, which are then further acted upon with the production of alcohol.

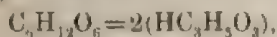
The following are the chief varieties of fermentation :

The Alcoholic Fermentation.—This is mainly brought about by the decomposition of sugars of the hexose group ($C_6H_{12}O_6$), principally dextrose and lævulose, by yeasts into alcohol and carbonic acid, but some of the bacteria and moulds also produce appreciable quantities of alcohol. Other carbohydrates by the action of enzymes secreted by the organisms may be converted into hexoses, which are then fermented. The general reaction is as follows :

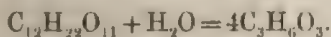


As a matter of fact small amounts of by-products appear in addition to the alcohol and carbonic acid, viz. glycerin, succinic acid, and higher alcohols. Until 1897 no enzyme had been obtained which would carry out this change; it only occurred when the living yeast-cells were present, but in that year Buchner by grinding up the living yeast cells obtained a juice which decomposed dextrose with the formation of alcohol and carbonic acid. This 'zymase' Buchner claimed to be the alcoholic enzyme of yeast.

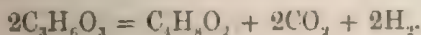
The Lactic Acid Fermentation.—This is brought about chiefly by bacteria. Hexoses are converted into lactic acid, the reaction being



but it is probably not actually so simple as this, for carbonic acid is given off at the same time. A familiar example of this form of fermentation is the souring of milk, in which the lactose is acted upon as follows:



The Butyric Acid Fermentation.—Butyric acid is formed from carbohydrates by the action of bacteria, mainly the *Bacillus butyricus* and *Clostridium butyricum*, the latter being an anaërobic organism, some by-products being formed in addition. Milk which has been just boiled usually undergoes the butyric rather than the lactic fermentation, the spores of the butyric organisms surviving. Lactic acid is first formed, and this is then converted into butyric acid:



The Acetic Acid Fermentation.—The conversion of alcohol into acetic acid is also due to bacteria, familiar examples of which are the souring of beer and wine.

The above are the more important fermentations brought about by micro-organisms, but there is no reason, except conventional usage, why pigment formation, nitrification, &c. should not be described as fermentations. It is uncertain whether the fermentive changes are brought about directly by the vital activities of the micro-organisms, or whether enzymes are first secreted. In a number of instances certainly the latter is the case, and the following are further examples of it :

1. Peptonizing zymolysis, e.g. the liquefaction of gelatin.
2. Invert zymolysis.
3. Diastatic zymolysis.
4. ' Fermentation ' of cellulose in the digestive tract—probably by secreted enzymes.
5. Curdling of milk by rennet-like enzymes.
6. ' Fermentation ' of urea by an enzyme secreted by the *Micrococcus ureæ* &c., with the formation of ammonium carbonate. These enzymes do not seem to possess any poisonous action.

Formation of Pigment.—Numerous organisms, especially those of air and water, during their growth produce various coloured pigments. They are termed chromogenic bacteria, examples of which are the *Sarcina lutea* and *Micrococcus cereus*, var. *flavus*, which form citron-yellow pigments ; the *Bacillus prodigiosus* and *Spirillum rubrum*, red pigments ; the *Bacillus violaceus* forms a rich violet one ; and the *Bacillus pyocyaneus*, a blue. A large number of chromogenic organisms require oxygen for the production of the pigment, and potato is often the most favourable culture medium. In some cases the medium may become coloured, and the property of fluorescence be conferred upon it, as is the case with the *Bacillus fluorescens liquefaciens*. Usually the pigment is extra-cellular, occasionally, as in *B. violaceus*, it is intra-cellular.

Phosphorescence or light-production is developed by

some bacteria, notably by many marine forms, and is well seen in decomposing fish. Some spirilla are also known occasionally to produce phosphorescence.

A Necrotic action on the tissues is produced by many pathogenic organisms. For example, the tubercle and glanders bacilli cause necrosis and caseation of the surrounding tissues.

Gas Production.—This is common to many organisms. The gas may consist of carbonic acid, hydrogen, or marsh gas, and in some cases of foul-smelling sulphur compounds, sulphuretted hydrogen, mercaptans, &c.

Sulphuretted hydrogen may be detected by the blackening of lead acetate paper. Methyl mercaptan may be detected by aspirating a current of air through the culture, through a calcium chloride drying-tube, and then through a test-tube or small flask containing isatin dissolved in concentrated sulphuric acid. The red colour of the isatin solution is changed to olive- or grass-green by the mercaptan.

Basic or Alkaloidal Bodies : Ptomines.—These are a very important group of nitrogenous bodies, analogous to the vegetable alkaloids and mostly solid and crystalline in nature, which are formed by the action of bacteria on protein and albuminoid matter. They often occur naturally in decomposing and putrefying food, meat, fish, &c., and as many of them are virulent poisons, they are of considerable practical import. Generally speaking, the poisoning due to tainted food is due to the absorption of toxic ptomines formed by bacterial action. A number of toxic ptomines were isolated by Brieger from cultivations of pathogenic microbes, and great importance was once attached to them. They are referred to in the chapters describing the pathogenic organisms.

Brieger's work, however, needs revision, for his methods were not such as to exclude alteration by the reagents employed.

MANUAL OF BACTERIOLOGY

enson obtained traces of a highly poisonous crystal-
mine from some sardines that had caused death.
n has isolated a body, tyrotoxinon, apparently
l with diazobenzene, from poisonous cheese and
Mytilotoxin ($C_6H_{11}NO_2$) is the specific poison of
mussels. The ptomines are thus of considerable
legal importance.

ic proteins are also formed by bacteria. Martin and
have described these as being allied to the proteoses.
nd Yersin suggested that the diphtheria poison
e an enzyme, while Brieger and Fränkel regard it
minous. These non-basic chemical poisons have
amed tox-albumins, and are considered to be the
toxic poisons of the pathogenic bacteria. In the
state of our knowledge it will be better, perhaps,
them simply 'toxins' (see p. 136).

LITERATURE.

trification, see Warington, *Journ. Chem. Soc.* 1886, et seq.

CHAPTER II.

METHODS OF CULTIVATING AND ISOLATING ORGANISMS.

It is necessary for the satisfactory study of micro-organisms in their relation to the various processes of infection and disease, of fermentation, putrefaction, and the like, to separate or isolate the different species in a mixture, and, having done so, to cultivate, grow, or propagate each species on suitable soils through successive generations. A slight consideration will show that unless we work with pure cultures—that is, cultures consisting of a single species—we can never be sure that a particular result is due to a given organism; in a mixture several or all of the forms present may conduce to the effect produced. With regard to the pathogenic organisms, or disease germs, Koch has laid down certain conditions which have been termed ‘Koch’s Postulates’ (p. 137), which must be complied with before the relation of an organism to a disease process can be said to be completely demonstrated, one of which is that ‘the organism must be isolated and cultivated outside the animal body on suitable media for successive generations.’

In order to isolate organisms in a state of purity it is absolutely necessary to employ vessels, instruments, and culture media which are sterile, that is, free from any living organisms, and to possess the means of manipulating them in such a way that the entrance of organisms from without is prevented, and contamination avoided. Various methods of destroying and of getting rid of organisms are known,

such as the use of chemical 'germicides,' heat, and filtration through porous porcelain. The addition of chemical germicides, such as carbolic acid or corrosive sublimate, is out of the question; for although the vessels and media might be rendered sterile thereby, the growth of the organisms which are being investigated would equally be prevented, so that the last two, viz. heat and filtration, are those which are employed, the former being used for

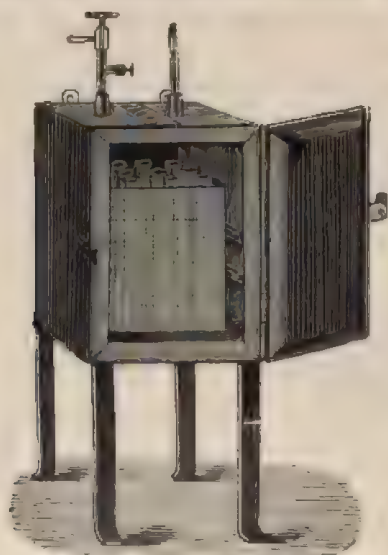


FIG. 1. HOT-AIR STERILIZER.

vessels, instruments, and culture media, solid and fluid, the latter for fluid culture media only.

Various apparatus are needed for sterilization and the preparation of culture media. These will now be described.

Hot-air Sterilizer (fig. 1).—This is a somewhat cubical box of sheet iron with double walls, having an air-space of nearly an inch between them, and furnished with a door. The bottom should be protected with a loose piece of sheet

iron which can be renewed as it 'burns' away. The top is perforated with a couple of holes, through one of which a chemical thermometer, registering to 200° C., is inserted in a cork, while through the other some form of mercurial regulator can be introduced if required, but is not usually needed. In the hot-air sterilizer all thin-glass vessels and cotton-wool are sterilized by heating to a temperature of about 150° C. by means of a Bunsen or a small ring burner under the sterilizer, which is supported on a suitable iron stand. If the sterilizer is placed on a table or other wooden support, a piece of sheet iron, asbestos cardboard, or uralite should be laid over the wood to protect it from the heat. An inexpensive substitute for the hot-air sterilizer may readily be devised, any iron box or even a biscuit-tin being used for the purpose.

Steam Sterilizer (fig. 2). -

This consists of a cylindrical or rectangular vessel of tinplate, galvanized iron, or copper, covered on the outside with a layer of felt or asbestos, having a false perforated bottom supported a few inches above the true bottom, and provided with a movable lid. In the steam sterilizer or 'steamer' the culture media, and thick glass vessels and other apparatus which would crack or be damaged by the high temperature of the hot-air sterilizer, are sterilized by steam. The lower chamber of the steamer, below the false bottom, is partly filled with water, which is boiled by means of a Bunsen or ring burner.



FIG. 2.—STEAM STERILIZER.

Above the false bottom the culture media or apparatus are placed, and are sterilized by the steam at 100° C. which fills this space.

Here again an inexpensive substitute may be devised; the ordinary kitchen saucepan with steamer will do well for many purposes, while a 'warren pot' answers admirably.

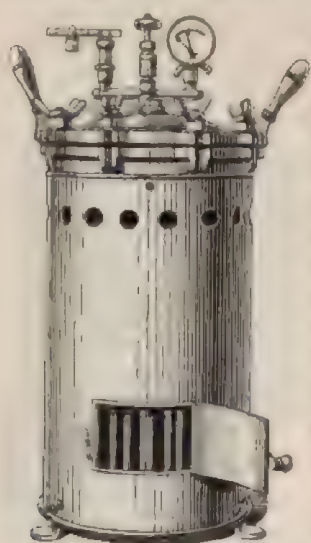


FIG. 3. — AUTOCLAVE.

Autoclave (fig. 3).—This is most useful for many purposes, but it is expensive and not a necessity, as the steam sterilizer can be made to answer almost every purpose for which the autoclave is employed with the expenditure of a little more time and trouble. It consists of a strong boiler of brass or gunmetal with a movable lid, which can be fixed down by means of screw-bolts. The lid is provided with a safety valve, a gauge for indicating the pressure and temperature, and a stopcock to relieve the pressure if required. A small quantity of water is placed in the bottom, and the

media or apparatus to be sterilized having been introduced, the lid is screwed down. It is heated by means of one or more Bunsen burners, which are turned down when the required temperature has been reached. The temperature usually employed is about 115° to 125° C. Care should be taken when heating fluids that the vessels are not filled too full, and that the autoclave is allowed to cool down to below 100° C. before relieving the pressure by opening the stopcock, or a good deal may be lost by violent ebullition. Also, while raising the temperature the stopcock should

always be left open until steam is being freely generated in order that the air may be expelled.

Air-pump.—An exhaust pump is very useful for many purposes, such as evaporating to dryness *in vacuo*, filtration through porous porcelain filters, &c. Any form will do, but of the more elaborate ones the Fleuss pump (fig. 4) made by the Pulsometer Engineering Company is by far the best. In using it care must be taken that no fluid or moisture gains access to the barrel; to avoid this it is a good plan to intercept the connecting pipe with a vessel containing strong sulphuric acid (D, fig. 4), over the surface of which the exhausted air has to pass. A double-necked Woulfe's bottle does well for this, the inlet and outlet tubes extending nearly down to, but not dipping below, the surface of the sulphuric acid.

For greasing the vessels &c. to make air-tight joints beeswax dissolved in the Fleuss pump oil with the aid of heat to a stiff paste is a good composition, or the resin ointment of the Pharmacopœia may be used.

Bell-jars with ground rims and one or two tubules are useful for evaporation *in vacuo*. They should stand on a square of thick ground glass. To make an air-tight joint the surface of the rim of the bell-jar, which must be quite clean, should be well greased and pushed thoroughly home on the ground-glass plate. A thick ridge of grease should then be plastered all round the angle formed by the rim of the bell-jar and the glass plate. Thick rubber pressure tubing must be used for connections, and all joints should be well greased. For evaporating large quantities of fluid the writer devised a copper stand with shelves, the shelves supporting glass dishes containing alternately strong sulphuric acid and the fluid to be evaporated, the whole being placed under a suitable bell-jar. A mercurial gage is a useful addition to show the amount of exhaust and the occurrence of leakage. The ordinary glass filter pumps

used in chemical work and actuated by a stream of water are also useful for many purposes.

Porous Porcelain Filters.—The two forms which are generally employed are the Pasteur-Chamberland and the Berkefeld. These consist of 'candles' composed in the former of unglazed porous porcelain, in the latter of a specially prepared diatomaceous earth. The filtration through the Pasteur-Chamberland is much slower than through the Berkefeld. Both give a germ-free filtrate, but



FIG. 4.—FLEUSS EXHAUST PUMP, ARRANGED FOR FILTRATION.

the latter should be employed if the fluid is thick or contains many particles; a preliminary filtration through paper is an advantage. A useful method of conducting filtration is the following. The filter 'candle' *B* (fig. 4) is connected by a short length of pressure tubing with a piece of glass tubing passing through a rubber cork in the neck of an ordinary filtering flask *C*. The 'candle' is placed in a jar *A*, such as a glass measure or urine-jar, which is filled up with the solution to be filtered. The lateral branch of the filter flask is then connected with the air-pump. On exhausting,

the fluid passes through the filter 'candle' over into the filtering flask, in which it is collected. Before use the 'candle' should be well scrubbed and some water or half per cent. carbolic run through to clean it, or the whole may be sterilized in the steamer for an hour or two. After use the same process should be repeated to cleanse it.

Flasks, Beakers, and Test-tubes.—A good supply of these is required of various sizes: Erlenmeyer and ordinary shapes, tall and short forms of beakers, &c. A few yeast flasks are also useful. These consist of an ordinary small flask, into the neck of which a piece of glass tubing is sealed at right angles, and turned down so that it extends nearly as low as the bottom of the flask (fig. 12, p. 67). Beakers and flasks of 'Jena' glass are to be preferred. Enamelled iron ware, jugs, saucepans, mugs, &c. may replace glass for many purposes.

The best size of test-tube is $6'' \times \frac{5}{8}''$; a few $6'' \times \frac{1}{2}''$ should also be kept.

Platinum Needles (fig. 5). Two or three platinum needles are required. They consist of about two inches of



FIG. 5.—PLATINUM NEEDLES.

platinum wire in a handle of glass rod. One end of a glass rod is softened in the Bunsen or blowpipe flame, and about an eighth of an inch of the platinum wire is embedded in it with a forceps, the wire having been first heated to a red heat. The glass-wire joint is then well annealed in the flame and allowed to cool slowly. Metal handles may also be used. Two thicknesses of platinum wire are desirable, viz. 0.4 mm. (27-28 B.W.G.) for most purposes, but a thicker wire of about 0.7 mm. where stiffness is

MANUAL OF BACTERIOLOGY

, and one or two 8 inches or more in length are

ps, Needles, &c.—Several forceps are necessary, ordinary dissecting form in two or three sizes, one or two of fine pointed, two or three small brass ones, and three pairs of the 'Cornet' pattern. A few ordinary needles of various sizes mounted in wooden handles for purposes.

s Pipettes and Capillary Tubes.—These are useful for serving or storing blood or pus, &c. for examination, for the water in making cover-glass specimens, and for other purposes. A piece of glass tubing is heated in a blowpipe flame until quite soft; it is then *taken out of the flame* and the two ends pulled steadily apart; this produces a capillary tube of greater or lesser length and smaller



FIG. 6. GLASS PIPETTE.

PREPARATION OF STERILE TEST-TUBES, FLASKS, &c. FOR THE
RECEPTION OR MANIPULATION OF CULTURE MEDIA.

To Sterilize Cotton-wool.—The best quality non-absorbent cotton-wool should be used for plugging purposes. The wool should be pulled apart so as to assist the penetration of heat; in the compressed condition the interior is difficult to sterilize. The separated wool is placed in the hot-air sterilizer and the temperature slowly raised to 145° C. and maintained at this for at least an hour. Above 150° C. cotton-wool becomes brown and brittle.

Glass Vessels.—The vessels (usually test-tubes, flasks, and dishes) are thoroughly washed and rinsed in water, then rinsed with 25 per cent. hydrochloric acid, and afterwards washed well with tap-water and drained. A final rinse with distilled water or alcohol is an advantage, as no deposit then occurs on drying. The cleansed vessels should be dried before sterilizing, either in the air or by placing in the hot air sterilizer for half an hour. When dry, the vessels are plugged with a firm plug of the sterilized cotton-wool, and are placed in the hot-air sterilizer, the temperature of which is then raised to about 150° C. They should remain at this temperature for not less than half an hour, after which the sterilizer and its contents are allowed to cool slowly.

If tubes or flasks are required in a hurry they may be rapidly sterilized as follows: After washing in water, they are rinsed with 5 per cent. carbolic, then with absolute alcohol, and finally with ether, and are then well flamed over a Bunsen flame, holding in a suitable forceps or holder. The ether evaporates and burns at the mouth, and when dry, a pledget of cotton-wool is held in the forceps and singed in the flame, and, while burning, the tube or flask is plugged with it.

MANUAL OF BACTERIOLOGY

dishes for plate cultures, graduated pipettes, &c. are sterilized as described for tubes and flasks. They may be sterilized and kept in sheet-iron or copper boxes of suitable size and shape.

In thick-glass vessels, such as measures &c., have to be sterilized, it is not safe to do this in the hot-air sterilizer. The heating and cooling are carried out very slowly, as they are very liable to crack. It is preferable, after cleaning, to wash them with sterilized cotton-wool and steam in the steam sterilizer for an hour on two successive days, raising the temperature and cooling slowly on each occasion.

CULTURE MEDIA.

The ordinary laboratory methods of preparing culture media are here given. 'Standard' media, having definite compositions, are now largely employed. For the method of sterilization see p. 56. Certain special media will be described later.

culture media, viz. peptone beef-broth, gelatin, and agar-agar, is an infusion of meat prepared usually from beef. In order to prepare this infusion, which may be termed acid beef-broth, proceed as follows: Take 1 lb. of beef ('gravy beef') free from fat, chop fine or mince, add 1 litre of tap-water, and allow it to simmer in a saucepan for one hour, cool, remove any solidified fat from the surface, and filter through filter-paper into a clean glass flask. If not required for immediate use, plug the neck of the flask with cotton-wool and steam in the steam sterilizer (or boil) for three-quarters of an hour on two successive days; it may then be kept until required.

Peptone Beef-broth.—Take 1 litre of the acid beef-broth, add to this 10 grams of peptone (Witte's) and 5 grams of common salt (i.e. 1 per cent. peptone and 0.5 per cent. sodium chloride), mix in a flask, and steam in the steam sterilizer until dissolved. When dissolved, remove from the steam sterilizer and render slightly alkaline with a 10 per cent. solution of caustic soda (preferably) or of sodium carbonate, *glazed* litmus-paper being used as an indicator.

Having done this, return to the steamer for one hour, then filter through two thicknesses of German filter-paper. It should now be quite clear and bright and may be kept in bulk, after sterilizing, or be introduced into test-tubes &c., and sterilized. Beef-broth if prepared in this manner may need no clarifying, but if it should filter at all cloudy, cool to 50° C., add the white of an egg beaten up with the shell,

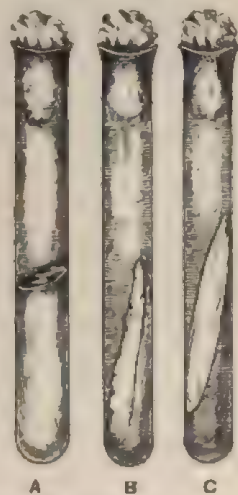


FIG. 7.—TUBES OF CULTURE MEDIA.

A, upright agar; B, potato;
C, sloping agar.

MANUAL OF BACTERIOLOGY

... for half an hour, filter, and finally sterilize as

... of meat infusion, meat extracts have been much used

The general opinion is, however, that meat-extract
are not such good nutrient soils for many purposes as
made from meat. The following is the composition of
broth:

Lemco	10-20 grams.
Peptone (Witte)	10-20 grams.
Sodium chloride	5-10 grams.
Water (preferably distilled)	1 litre.

constituents are dissolved with the aid of heat, neutralized,
and filtered. Lemco may also be used to make all the
media for which acid beef-broth is employed.

Veal-broth.—For some purposes veal presents advan-
ter beef, as for growing the tubercle bacillus. When
from the butcher's the veal is frequently powdered

Beer-wort.—Procure beer-wort (preferably unhopped) from the brewery. Allow it to stand in a cool place for twelve hours, filter, and then steam for an hour and filter again. Fill into sterile test-tubes and sterilize.

Glucose Broth.—For the cultivation of anaërobic organisms the addition of 0·5 to 2 per cent. of grape sugar is an advantage. It should be added after filtration.

Nutrient Gelatin.—Take 1 litre of the acid beef-broth in a large flask and add to it 100 grams of the best 'gold label' gelatin, 10 grams of peptone, and 5 grams of common salt. Place in the water-bath or steamer until quite dissolved. Then render faintly alkaline, as for the peptone beef-broth; cool to 50° C., and add the white of an egg, stir well, and return to the steamer for one hour. Filter through two thicknesses of filter-paper in a hot-water funnel (this is best, but it may be done in the steamer at a low temperature, i.e. 35° C.) Fill into test-tubes and sterilize. After the third steaming the tubes are allowed to solidify, either in the upright or oblique position, according as they are required for stab or surface cultivation.

In hot summer weather 15 or even 20 per cent. of gelatin (150 grams or 200 grams to the litre) are necessary for the product to remain solid, as nutrient gelatin melts at 24° C. or a little under. Prolonged boiling diminishes and ultimately destroys the gelatinizing power of gelatin, so the less it is heated the better. It must not be autoclaved.

Glucose Gelatin.—Ordinary gelatin with the addition of 1 to 2 per cent. of grape sugar.

Beer-wort Gelatin.—This is one of the best culture media for yeasts and some of the fungi (e.g. ringworm). Procure from the brewery some beer-wort, preferably unhopped, and add to every litre 100 grams of gelatin. Dissolve, clarify, and filter, as in the case of ordinary gelatin. It is not neutralized.

MANUAL OF BACTERIOLOGY

nutrient Agar-agar. This is one of our most valuable culture media, and has the advantage over nutrient broth that it remains solid at blood-heat.

Agar is a carbohydrate substance of high melting-point and considerable gelatinizing power, obtained from Eastern seaweeds. The powdered form is now generally used. Add 10 grams (i.e. 1½ per cent.) of powdered agar to 1 litre of beef-broth, together with 10 grams of peptone and 5 grams of common salt in a large glass flask, place in the water-bath until dissolved (half an hour to one hour), and render alkaline as for peptone beef-broth; allow it to boil at 50° C., and add the white of an egg. Return to the water-bath for an hour and a half, then filter through an *Aschmann* filter-paper ('papier Chardin') in a hot-water funnel over a steamer. By this treatment a litre of agar should pass through the filter in two to three hours. If it does not come through clear, add another white of egg and repeat the process.

When an autoclave is available a quicker and better method

Glycerin Agar.—Add 4 to 6 per cent. of glycerin to the nutrient agar after filtration and proceed as before.

Glucose Agar.—One or two per cent. of grape sugar is added to the nutrient agar after filtration.

Litmus Media.—The addition of neutral litmus to the various culture media is a useful method of demonstrating the production of acid or of alkali by organisms. To prepare the litmus solution take the lump litmus, powder finely, and boil with distilled water so that a saturated solution is obtained. Filter, and preserve in a flask stoppered with cotton-wool, after sterilizing by boiling for half an hour on two successive days. For some purposes a special solution of litmus, the Kubel-Tiemann solution, which can be procured ready for use, is employed. It must not have any antiseptic added to it (as is sometimes done to preserve it for use in the chemical laboratory).

Sufficient of this litmus infusion is added to the nutrient media, after filtration, to tinge them a distinct purplish colour. After steaming the colour has usually disappeared, but returns as the tubes cool.

Milk.—If possible, procure separated milk, but failing this, take ordinary new milk, place in a tall cylinder or beaker, and allow it to stand overnight in a cool place, preferably in an ice safe. Then pipette off the milk from the bottom, rejecting the cream. Introduce into test-tubes to the depth of about an inch to an inch and a half and steam for one hour on two successive days. The milk is usually tinged with litmus before tubing, forming *litmus milk*.

Potatoes.—Choose sound potatoes, and scrub them well with water to remove dirt. Cut off the ends, and with a cork-borer, slightly smaller than the test-tubes which are used, bore through the potato so that a cylindrical piece is removed. Push this out of the borer, and divide it into two portions by a very oblique transverse cut, so that two

MANUAL OF BACTERIOLOGY

shaped pieces are obtained, and in this manner prepare many pieces as there are tubes to be filled. Place them in a basin under the tap, and allow the water to flow over them for about two hours. This prevents the darkening of the potato in the subsequent steaming. The test-tubes or the potato-wedges are prepared as follows: After plugging and sterilizing in the ordinary way, introduce a small pledget of sterilized wool into each, push to the bottom, and moisten with a little sterilized distilled water. Drop the potato-wedges into the tubes, plug, and sterilize by steaming for three-quarters of an hour on two successive days (fig. 7, b). The object of the moist wool is to prevent drying, and for the same purpose Roux's tubes (fig. 8) may be used, the lower bulb being filled with water.

Blood Serum.—Clean some glass jars of about 2 to 3 litres capacity, plug with wool, and sterilize in the steamer for an hour or

The serum inspissator is practically a large incubator (see p. 58) with inclined shelves, on which the tubes of serum rest.

Löffler's Blood Serum.—The serum is procured in the manner described for the simple blood serum. To every three parts of serum one part of sterile glucose-broth is added, mix, fill tubes, and inspissate as before.¹

Serum, ascitic and hydrocele fluids, &c. may be preserved in bulk and used as required. The material is collected as aseptically as possible, 5 per cent. chloroform added, the whole well mixed, and kept in a cool place in the dark in a well-stoppered bottle. During the process of sterilization the chloroform is volatilised.

Fluid Serum &c.—Fluid blood serum, ascitic and hydrocele fluids, &c. are sometimes useful, and may be used alone or mixed with peptone beef-broth in various proportions.

Ascitic or hydrocele fluid may be obtained by using sterile trocars &c., and carrying out the tapping with aseptic precautions, collecting the fluid in sterilized flasks. It is better to collect in several small flasks than in one large one.

Fluid blood serum may be obtained by collecting blood with aseptic precautions in sterilized flasks. When the blood has coagulated and the serum separated, the serum is pipetted off with a sterile pipette into sterile flasks.

The flasks of serum &c. should be kept in a warm place for two or three days to make sure that they are sterile, those in which a growth appears being rejected.

Serum, ascitic fluid, &c. may also be obtained sterile by filtering through a sterilized Berkefeld filter into sterile flasks.

Serum Agar (Kanthack and Stevens).—Ascitic, pleuritic, or hydrocele fluid is collected in clean (not necessarily

¹ Löffler originally used ox serum.

MANUAL OF BACTERIOLOGY

ed) flasks, and allowed to stand overnight in a cool place to allow the sediment or blood to collect. The clear fluid is then poured off, and to each litre enough of a 10 per cent. caustic potash solution is added to render it very strongly alkaline—usually about 2 c.c. to every 100 c.c. of fluid. The alkaline fluid is heated in the autoclave for four hours. To this fluid 1·5 to 2 per cent. of agar is added, and the mixture heated until the agar is dissolved. It is then filtered, introduced into test-tubes, sterilized, and solidified in the ordinary way. The addition of 5 per cent. of glycerin and 1 per cent. of glucose is an advantage. Serum agar may also be prepared by adding sterile serum or hydrocele or ascitic fluid, warmed to 45° C., to nutrient agar (2 to 3 per cent. agar) melted and cooled to 45° C. Equal parts of the serum and agar may be used, or 1 part of serum to 2 parts of agar.

Sloped Agar.—This may be prepared by smearing the surface of the agar in sloping agar tubes with blood obtained

washing with carbonate of soda solution, rinsing in sterile water, soaking in 1-500 corrosive sublimate solution, and washing in alcohol and in ether. A small hole is then chipped in the shell with a sterile needle and the inoculation made through this. The hole is afterwards closed with a little sterilized wool and collodion.

Uschinsky's Fluid.

	Parts
Sodium chloride	5-7
Calcium chloride	0.1
Magnesium sulphate	0.2-0.4
Di-potassium phosphate	2-2.5
Ammonium lactate	6-7
Sodium asparaginate	3-4
Glycerin	30-40
Water	1000

A solution of known composition without protein which can be used for investigating the chemical products of bacteria. Pathogenic organisms grow well in it and produce their toxins.

Pasteur's Fluid.

	Parts.
Cane sugar	10
Tartrate of ammonia	1
The ash of 1 gramme of yeast	—
Water	100

A good culture fluid for yeasts &c.¹

STANDARD NUTRIENT MEDIA.

Slight variations in the composition of the nutrient media have a marked influence upon the characters of the growths of micro-organisms developing upon them. In

¹ Several formulæ for synthesised media will be found in the *Journal of Experimental Medicine*, iii. p. 666.

MANUAL OF BACTERIOLOGY

to obtain more uniformity for descriptive purposes &c. The Committee of the American Public Health Association has adopted a scheme for the preparation of nutrient media of uniformly constant composition and reaction. Eyre¹ has devoted considerable attention to this subject, and the following descriptions are based largely upon his papers.

Preparation of Acid Beef-broth.—1000 c.c. of distilled water are introduced into a large flask, 500 grams of minced fresh lean beef added, and the mixture is placed in a water-bath at 40°–45° C. for twenty minutes with frequent agitation. It is then boiled for ten minutes, cooled, and filtered through paper. To the filtrate sufficient distilled water is added to make up to 1000 c.c.

Standardization.—This may be most simply determined in the case of acid broth. A 100 c.c. Erlenmeyer flask is rinsed out with boiling distilled water, 25 c.c. of the acid beef-broth are introduced into it, and 0.5 c.c. of phenolphthalein solution is added (0.5 per cent. phenolphthalein in 95 per cent. alcohol). This is kept boiling and decinormal

litmus but neutral to phenolphthalein. To reduce the alkalinity (to litmus) normal hydrochloric acid is then added. The American Committee recommended an acidity of $+1.5$ —that is, to every 100 c.c. of the medium neutral to phenolphthalein 1.5 c.c. of the normal hydrochloric acid are added. Eyre advises a reaction of $+1.0$ (i.e. 1 c.c. of normal hydrochloric to every 100 c.c.), while Chester considers that the acidity should not exceed $+0.5$. Whatever the reaction adopted, it should be stated. Similarly, if a medium is used which is alkaline to phenolphthalein, this is expressed by the minus sign; e.g. a reaction of -1.5 indicates that to every 100 c.c. 1.5 c.c. of normal hydrochloric acid must be added to render it neutral to phenolphthalein, or, what is almost (but not quite) the same thing, that to the *neutral* medium 1.5 c.c. of normal caustic soda solution have been added to every 100 c.c. Various methods are adopted to obtain the standard reaction; the American Committee recommend first neutralizing and then adding sufficient acid (or alkali); Eyre, having calculated the acidity, adds only sufficient alkali to *reduce* the reaction to the required point. Eyre describes the reaction as represented by the number of c.c.'s of alkali or acid per litre, e.g. $+10$ on Eyre's scale is equivalent to the American $+1.0$. In making nutrient broth, agar and gelatin, the salt and peptone, and agar or gelatin, are added and dissolved, and the titration and neutralisation carried out as described, on the fluid medium itself, and after neutralization the whole is heated over a water-bath for half an hour before filtration.

THE CULTIVATION AND ISOLATION OF MICRO-ORGANISMS.

It should be clearly understood that micro-organisms cannot usually be identified by their microscopical characters alone. We can state from a microscopical examination the form of an organism, that it is a *bacillus*, or a *micrococcus*,

MANUAL OF BACTERIOLOGY

reina, its size, that it is motile or non-motile, sporing or non-sporing, but we cannot as a rule go beyond this. It is necessary in most cases to ascertain the characters of the various organisms on the various culture media before they can be identified, and this is the main reason for using a varied assortment of nutrient soils. It is likewise necessary for the successful cultivation of pathogenic organisms, i.e. those connected with disease processes and acting in or upon the bodies of man and of animals, to maintain the cultures at a temperature approximating to that of the host. For this purpose some form of incubator is required. This consists of a box or chamber of copper or other metal with double walls (fig. 9), the space between which is filled with water, the outside being covered with wood or some other non-conductor. The water between the walls is heated by means of a small burner, the gas supply of which passes through some form of regulator inserted in the water, so that the temperature, indicated by a thermometer inserted through a hole in the top, can be

an advantage. If there be only one, the regulator should be set for a temperature of 37° C.; if more, another should be kept at about 22° C. The incubator at 37° C. is termed the warm or blood-heat, and that at 22° C. the cool or room temperature one. An ordinary

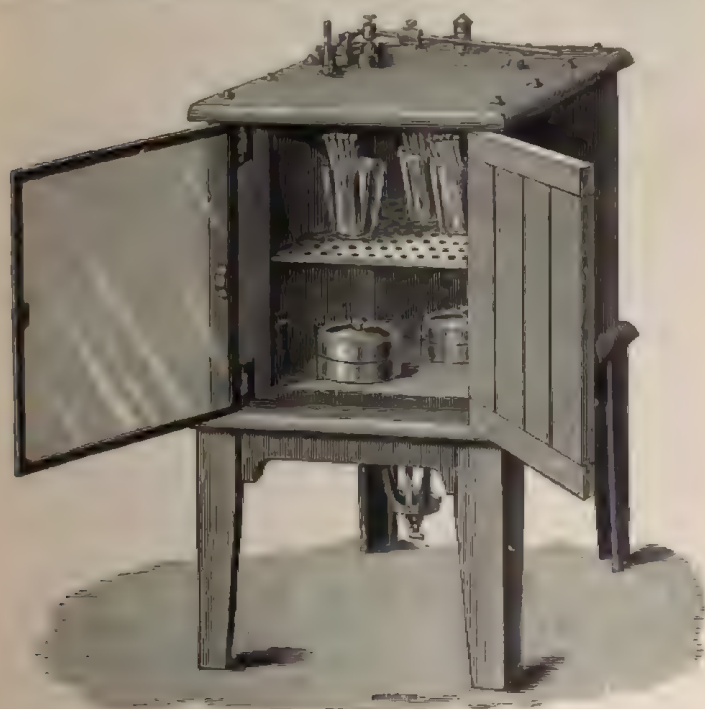


FIG. 9.—HEARSON'S INCUBATOR.

warm room will answer most of the purposes of the cool incubator. A third incubator set for 42° C. is useful for water work.

A substitute for the large and expensive incubator can readily be devised. An ordinary chemical hot-water oven may be employed, or simply a smaller tin set in a somewhat

MANUAL OF BACTERIOLOGY

ne, the interspace being filled with water ; while, by little scheming, regulators can be dispensed with by the use of a small gas or lamp flame, varying its size at distance from the bottom until the right temperature is attained. Gas is certainly a great convenience, where none is available, regulating oil lamps can be used to take its place. Electricity has also been used for heating incubators.

Gelatin will remain solid only at temperatures below 30° C. and cannot therefore be placed in the blood-heat incubator without becoming for practical purposes a fluid medium.

Agar, however—and this is one of its most valuable properties—does not liquefy below a temperature of 100° C., though when once liquefied it does not set again until the temperature has fallen to about 45° C. Gelatin is therefore usually reserved for use at low temperatures, while agar, blood serum, potato, and the fluid media can be used differently either at low or at high temperatures. Agar is often a better cultivating medium than gelatin, even

the formation of a clot of casein. Most organisms which liquefy gelatin coagulate milk, but the converse is not the case. Agar is carbohydrate, not albuminoid, in nature, and is not liquefied by any organism. In fluid media, such as broth and peptone water, chemical tests can be applied, especially for indole, which is formed by some organisms but not by others.

Method of Inoculating Tubes.—Supposing there is a pure culture in a test-tube from which subcultures are desired, the following is the method of procedure. Tubes of suitable media having been selected, they are placed in a test-tube rack. Their mouths are then singed by holding in the Bunsen flame for a few seconds, and with a forceps, also sterilized by heating in the flame, the wool plugs are loosened by a rotatory motion, and then partially withdrawn. A platinum needle is selected and carefully straightened. The original culture-tube having been singed and its plug partially withdrawn, in the same manner as the sterile tubes, is then taken in the left hand between the thumb and index finger with the palm upwards, and is held obliquely, the mouth of the tube pointing to the right, a tube of sterile medium being held side by side with the original culture in an exactly similar manner. The wire of the platinum needle is then heated to redness by holding nearly vertically in the flame, and the lower part of the handle is also heated carefully. Holding the sterilized needle between the finger and thumb of the right hand, the plug of the original culture is now withdrawn by grasping between the ring and little fingers of the right hand, and is held there while the platinum needle is carefully introduced into the tube without touching the mouth or sides, and a trace of the growth is picked up with it, preferably from the margin. To be sure that the needle is cool, it may be first touched on the medium where there is no growth. The needle is quickly withdrawn without touching

MANUAL OF BACTERIOLOGY

of the tube and the plug at once replaced. The plug of the sterile tube is now withdrawn in the same manner, and the inoculated needle introduced. If a typical surface culture is desired, a single light streak is made with the needle from the bottom to the top of the medium without touching the surface; if an abundant growth be required for any purpose the whole surface of the medium is rubbed with the needle; if a stab culture, the needle is pushed steadily into the centre of the medium and withdrawn. If a fluid one, the growth removed is rubbed up on the inner surface of the tube at the margin of the fluid, and the surface is washed down by tilting the tube. The inoculation being completed, the plug is quickly replaced, and the tube is again heated in the flame to destroy the remainder of the growth upon it. If the original culture is in a solid, or a fluid medium, a looped platinum needle sometimes be used with advantage. The inoculations being finished, the mouths of the tubes are singed and the wool is pushed in level with the lip. Before replacing the

for five minutes, and then cooled and solidified in cold water. The object of this is to soften the medium so that it does not split, as a dry medium will, when the needle is plunged into it, while the needle track closes up, and the dissolved oxygen is got rid of. The tubes being cool, the inoculation is made with a long thin needle, preferably straight, but in some cases a closed loop will pick up more material. It is inoculated and plunged steadily into the centre of the medium, nearly to the bottom, rotated, and then withdrawn, and the wool plug replaced and singed. The tube is then carefully heated at the upper border of the medium so as to melt this slightly and seal the puncture, and a well-fitting rubber cap is applied while the tube is hot. The heating expels a portion of the air and, with a well-fitting cap, creates a negative pressure within the tube, so that the residual oxygen is not so readily absorbed, or the tubes may be placed in a Buchner apparatus (see below). The tubes are placed in the incubator at a suitable temperature, and it will be found that the most strictly anaerobic organisms can be cultivated in this way.

When, however, an organism is required to grow anaerobically on the surface of the medium, or in a fluid medium, some other method must be adopted. The tubes may be placed under the receiver of an air-pump and exhausted as completely as possible. This is not very convenient, for it is difficult without great care to maintain a vacuum, and special receivers must be used when the cultures have to be incubated at blood-heat, while with fluid media ebullition causes considerable difficulty.

For fluid cultures, Hamilton's method is the simplest of all. The fluid in the tubes is covered with a layer of olive oil 1-2 cm thick and the tubes are then sterilized. The layer of oil prevents the access and entrance of oxygen. The only disadvantage is that the inoculation, or the withdrawal of culture, must usually be performed with

MANUAL OF BACTERIOLOGY

glass pipette ; if a needle be used the material is able to be detached in the oil.

Another method (Buchner's) is that usually adopted, consists in absorbing the oxygen by means of alkali pyrogallic acid, and so cultivating in an atmosphere of H_2 . This can be carried out in two ways—either in a wide-mouthed bottle with well-fitting glass stopper, sufficiently large to contain the test-tubes, or in a Buchner's tube. For the first, the stopper of the bottle is vaselined and the inoculated test-tubes are placed in it. Some solution of pyrogallic acid in water is run into the bottle by means of a thistle funnel, and then some caustic potash solution. A strong solution of pyrogallic acid is employed, and mixed with an equal volume of 20 per cent. caustic potash.¹ As quickly

about an inch and a half from the bottom. The constriction supports the test-tube culture, while the mixture of pyrogallie acid and caustic potash fills the portion below the constriction. A well-fitting rubber cork closes the mouth of the tube, and the joint may be paraffined for additional security.

The displacement of the atmosphere by means of hydrogen may be adopted, and is to be preferred for fluid cultures. Hydrogen does not seem to inhibit the growth of any anaerobic organisms, whereas carbon dioxide gas, which might be still more conveniently used, has a very decided inhibitory action on some species. The hydrogen is best generated from zinc and sulphuric acid in a Kipp apparatus, or the compressed gas in cylinders, or even coal-gas, may be used. Care must be taken that all joints are tight, and they may be paraffined with advantage. The gas should be passed through a strong solution of caustic potash, and may be passed through some alkaline pyrogallie acid if the most rigorous condition of anaerobiosis is desired, but for ordinary purposes this is not essential; it should also pass through two or three fairly firm plugs of cotton-wool to remove organisms; these must be dry, for if moist the passage of the gas may be stopped.

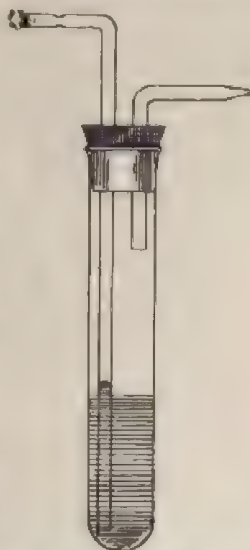


FIG. 11.—FRÄNKL'S TUBE FOR ANAEROBIC CULTIVATION.

For tube cultures Fränkel's method may be adopted (fig. 11). The broth or gelatin is introduced into a large strong test-tube, the mouth of which is plugged with

MANUAL OF BACTERIOLOGY

r cork pierced with two holes. Through these holes
ces of glass tubing pass, one to the bottom of the
e other just through the cork. Outside the cork
ubes are bent over at right angles and each is
slightly out so as to contract its lumen at about
dle. The long tube is connected with the hydro-
ply, and a current of the gas is passed through and
by the shorter tube. After the gas has been passing
ty minutes to half an hour, and *all oxygen has*
exhausted, the distal, i.e. shorter, tube is sealed off at
tracted portion in the Bunsen or blowpipe flame,
n the proximal or longer one in the same manner.
uber cork must, of course, fit well, and the joints
be paraffined. If gelatin be the medium, it must
fluid in a bath of warm water while the hydrogen
ng.

broth or fluid cultures, which are essential for
g toxic products, flasks are used which are fitted

writer. 'Yeast flasks,' which can be obtained in various sizes, are made use of, and are filled three parts full with a 2 per cent. grape-sugar bouillon. The neck is corked with a perforated rubber cork (A, fig. 12), through which a glass tube, B, passes to the bottom of the flask, projecting two inches above the rubber cork and here plugged with cotton-wool. The lateral tube of the yeast flask is also plugged with cotton-wool, care being taken that the plugs are loose enough to allow air to pass freely. The whole is sterilized and inoculated. The glass tube, B, which passes through the rubber cork, is then connected with a Kipp or other hydrogen-generating apparatus by means of a rubber tube, and a current of hydrogen is passed through the flask. The hydrogen bubbles through the bouillon and escapes by the lateral tube. After the gas has been passing for half an hour a small tube containing mercury, C, is applied to the end of the lateral branch, so that the

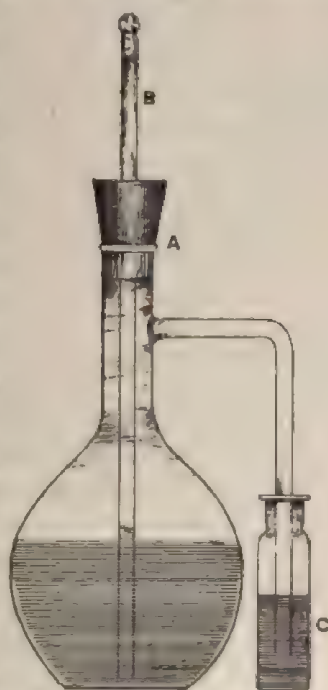


FIG. 12. YEAST FLASK ARRANGED FOR ANAEROBIC CULTIVATION.

open end just dips below the surface of the mercury, and the tube, B, which passes through the rubber cork, is sealed off in the blowpipe flame, care being taken that all the air has been expelled from the flask by a free current of hydrogen. The flask, with the capsule of mercury applied to the end of the lateral branch, can then be placed in the incubator.

MANUAL OF BACTERIOLOGY

The mercury forms a valve through which air cannot enter, while gases formed by the growth of the organism can exit.

In large flasks, the lateral tube may be just bent down and a little capsule of mercury hung on.

The addition of $\frac{1}{2}$ to 1 per cent. of sodium formate to culture media much simplifies anaërobic cultivation; *Clostridium* bacillus, for example, can be grown in formate in a stoppered bottle without any elaborate preparation for excluding the last traces of air. The sodium should be added immediately before the last inoculation, not previously, or decomposition may occur. Sodium sulphindigotate (0.3 per cent.) may be similarly

used in such a broth, Dean's bottle may be used for anaërobic cultivation. This consists of a bottle around the neck of which a gutter for mercury is formed. A loose stopper fits over the mouth of the bottle, and its edge

the organisms to be evenly distributed throughout the fluid. By transferring this volume to tubes of sterile media, pure cultivations could in some cases be obtained, a single organism having been sown in a tube.

It is obvious, however, that this method is at best an uncertain one, but the plate-culture method to a large extent obviates this uncertainty. It depends upon the following principles: In gelatin and agar we have media which, when melted, are fluid above 40° C. and solid at lower temperatures. By inoculating the fluid gelatin or agar, thoroughly mixing, and then pouring on to a level sterilized surface, so that the medium solidifies in a thin film, the organisms, wherever they may be situated, are fixed and are unable to wander, and, being in a good nutrient soil, grow and multiply and ultimately form a visible growth or colony; and each colony having arisen from a single organism, the growth is pure, and pure cultures can be obtained by inoculating tubes of sterile media from them.

When suitable, sterile nutrient gelatin is usually employed for the preparation of plate cultivations, as it is more easily manipulated than agar. Three tubes of sterile nutrient gelatin are melted at a low temperature in a beaker of water (gelatin melts at 24° C.; the temperature should not exceed about 45° C.) The tubes may be termed respectively 1, 2, and 3. Tube No. 1 is inoculated, by means of a platinum needle, with a trace of the growth from which pure cultivations are desired. The trace of growth is thoroughly mixed up and distributed throughout the melted gelatin; but if this mixture were poured on to a sterile level surface so many organisms might be present in the film that the colonies which developed would not be separate, but would form a confluent growth. To obviate this difficulty a second and a third dilution are prepared. The second dilution is made by inoculating the tube of melted gelatin No. 2 with one platinum loopful from tube

MANUAL OF BACTERIOLOGY

and thoroughly mixing up; and to be quite sure the resulting colonies will be isolated from each third dilution is prepared in the same manner isolating the tube of melted gelatin No. 8 with two platinum loopfuls from tube No. 2. In each case, organisms having been distributed throughout the by rolling and gentle shaking, the wool plug is withdrawn from the mouth of the tube, the mouth of the tube held in the Bunsen burner to prevent contamination, for a few seconds, and the melted gelatin poured on a sterile glass surface. Formerly plates of glass (hence the name); but now shallow glass dishes, about three or four inches in diameter, known as Petri dishes (fig. 19), are almost always employed. They are previously sterilized in the hot-air sterilizer in suitable iron or copper boxes holding



colonies are an aid in diagnosis and enable the various species to be picked out from a mixture. The colonies in gelatin are as a rule much more distinctive than those in agar. Whereas the plate cultivation prepared from tube No. 1 is generally too crowded, plates 2 or 3, or both, can be made use of, and it is apparent that, to make certain of isolating all the organisms from a mixture, several sets of plates should be prepared. Flat bottles (fig. 14) may likewise be used for plate culturing, and are also very useful for growing organisms in bulk for the examination of the constituents and actions of the bacterial cells.

In addition to the isolation of species from mixtures and for diagnosis, plate cultures are also used to enumerate organisms. Assuming that every colony arises from a single organism, which is approximately the case, the number of colonies represents the number of organisms originally introduced into the gelatin, and if a known weight or volume of the material inoculated be used, the number of organisms in it can be calculated. For example, in the bacteriological examination of water a measured volume of the water is added to melted gelatin by means of a sterilized pipette, and by counting the resulting colonies the number of organisms originally present in 1 c.c. of the water can be estimated.

Agar plate cultures may be prepared in a similar way. The agar, however, must be brought to a temperature of nearly boiling before it melts; it is then allowed to cool to 45° or 40° C. and the tubes inoculated in the same manner as for a gelatin plate culture described above. Unless the manipulations be carried out expeditiously the agar will solidify, or the agar film in the Petri dish be lumpy.

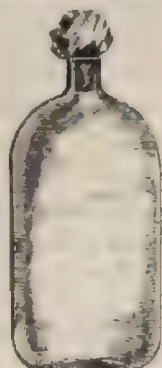


FIG. 14.
· PLATE · BOTTLES.

MANUAL OF BACTERIOLOGY

plates should usually be inverted during incubation, the growth may become confluent owing to the diffusion of water carrying the organisms all over the

The plate-culture method can be modified to suit particular circumstances: for example, the melted gelatin or agar inoculated, may be poured into the dishes and allowed to solidify, and the film then inoculated by streaking with the material, or by pouring a few drops of the material containing the organisms upon it. This is practically the way in which blood serum can be used, the sterile serum being placed in the Petri dish, solidified in the water bath, and then inoculated in the same manner as for blood-serum tubes, the solidified film inoculated.

For many purposes plates are unnecessary, the same results being obtained by rubbing over the surface of two or three slides of sloping agar or gelatin successively the *once* with the needle, straight or looped. In the second or third

colonies then develop in the film of gelatin, which is quite analogous to a film in a Petri dish.

Anaërobic Plate Cultivations are sometimes required. The plate cultures after preparation as described above can be placed in the receiver of an air-pump which is exhausted, and the colonies develop *in vacuo*, or the film in the Petri dish may be covered with a piece of sterilized mica well pressed down, or with a layer of sterilized oil, or pure liquid paraffin. In Botkin's method a bell-jar standing in a glass dish is made use of. The Petri dishes are placed on a support within the bell-jar, and mercury or oil is poured into the glass dish. By means of a piece of bent glass tubing a stream of hydrogen is passed into the bell-jar under its rim so as to displace the air, which bubbles out through the oil or mercury. When the air has been entirely displaced the glass tube is removed, the bell-jar weighted, and the whole placed in the incubator. Bulloch's apparatus is somewhat similar to this. Wide-mouthed jars with well-ground glass lids, which are luted down, are very convenient, the oxygen being absorbed with alkaline pyrogallol placed at the bottom, and the Petri dishes stacked on a glass capsule or other support to raise them above the fluid.

Double glass capsules may also be used. The plate culture is made in the smaller capsule, which is then inverted over the larger one, in which a little alkaline pyrogallol is placed, some oil being run over the surface of the pyrogallol on the *outside*, so that absorption from the external air is prevented.

The Esmarch roll cultures can be adapted for anaërobic plate cultures. The wool plug is replaced by a rubber cork with two holes in it, through which inlet and outlet glass tubes pass, as in Frinkel's anaërobic tubes (fig. 11). The roll culture having been prepared, and the film set, hydrogen is passed in and the tubes sealed off, or, better still, the

MANUAL OF BACTERIOLOGY

en is allowed to bubble through the inoculated melted the test-tube meanwhile being kept in a little warm to prevent the gelatin from solidifying, the tubes off, and the roll culture then prepared.

the detection of fermentation and gas production, cultures in glucose agar or shake cultures in gelatin may be employed. For the latter a tube of gelatin¹ is melted at a low temperature, inoculated with the organism, and allowed to solidify in the upright position; the organism is thereby distributed throughout the medium. Fermentation with gas production is indicated by the presence of gas bubbles, or even by the disruption of the medium. Durham's fermentation tubes are very convenient for showing fermentation. These are test-tubes containing suitable fluid media (10 c.c. each)

are peptone broth, the acid beef-broth for which has been treated with the colon bacillus (see p. 25), 1-2 per cent. peptone water, or a medium which has been largely used by Houston, Gordon, and others, consisting of distilled

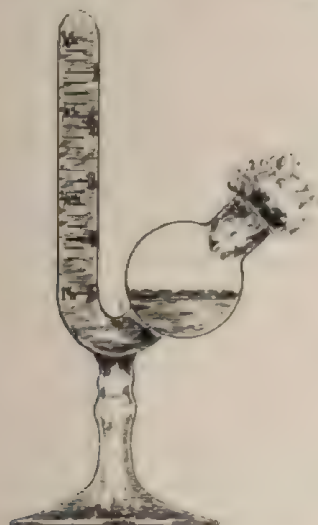


FIG. 16. —EINHORN'S SACCCHARIMETER.

water containing 'Lemco' 1 per cent., peptone 1 per cent., sodium bicarbonate 0.1 per cent.; to either medium is added 1-2 per cent. of glucose, lactose, saccharose, starch, inulin, mannitol, dulcitol, &c., and the mixture is tinged with litmus.

MANUAL OF BACTERIOLOGY

CHAPTER III.

PREPARATION OF TISSUES AND ORGANISMS FOR STAINING
AND MOUNTING. STAINING AND STAINING METHODS.

Numerous methods for preparing and staining tissues, organs, &c., have been published, but only a selected few of the generally useful will be given here. Special methods occasionally employed will be described when required.

PREPARATION OF TISSUES.

The demonstration of the bacteria in the tissues is of

(b) Place in methylated spirit 1 part, water 2 parts, for twenty-four to forty-eight hours, transfer to methylated spirit and water, equal parts, then to methylated spirit, and finally to absolute alcohol for like periods.

(c) Place in rectified spirit (86 per cent. alcohol) containing 1 per cent. of corrosive sublimate for twelve to forty-eight hours, and pass through increasing strengths of alcohol as in (b).

(d) Place for six to twelve hours in a saturated aqueous solution of corrosive sublimate. This is prepared by saturating boiling distilled water with the corrosive sublimate, cooling, and filtering. Keep in the dark. When removed from the corrosive sublimate solution the tissues must be washed in a stream of running water for an hour, or, better, placed for a day in 70 per cent. alcohol deeply coloured with iodine, to remove the excess of corrosive sublimate and prevent precipitation. The tissues are then passed through increasing strengths of alcohol, as in (b).

(e) Formalin, a 40 per cent. aqueous solution of formic aldehyde, is an excellent fixing agent. A solution of 1 part of formalin and 9 parts of water, or better physiological salt solution, may be used, the pieces of tissue remaining in this for twelve to twenty-four hours. They are then washed in running water for an hour or two and passed through increasing strengths of alcohol, as in (b).

All tissues after fixing and hardening should be preserved in dilute methylated spirit - water 1 part, methylated spirit 2 parts.

The methods (c), (d), and (e) are to be recommended, especially the last two, as the tissue elements are well fixed

be obtained in quantities of five bulk gallons, 'for scientific purposes only,' by special order from the Inland Revenue Authorities, Somerset House, W.C. If it cannot be procured, absolute alcohol must be employed. Duty-free absolute alcohol can also be obtained under somewhat similar conditions and is much cheaper than the ordinary.

MANUAL OF BACTERIOLOGY

In all cases the fixing fluid should be used in considerable excess. Fixing fluids containing potassium bichromate (as in Müller's fluid) and chromic acid seem to prevent the bacteria from staining with any certainty, and should be avoided.

SECTION CUTTING.

In order satisfactorily to demonstrate bacteria in tissues, and their relation to the tissue elements, it is usually necessary to prepare sections. For this purpose either the freezing or the paraffin method should be employed.

Freezing Method.—The tissue, in suitable pieces, must first be soaked in water to remove the alcohol. A convenient way of doing this is to place the material in a luted bottle, into the mouth of which an ordinary funnel is introduced, and the bottle with the funnel held under a stream of running water. The water passing through the funnel fills the bottle and overflows at the neck of the bottle and the sides of the funnel.

Add a piece of thymol or a little carbolic acid to prevent decomposition. Hamilton saturates the solution with boric acid, which does very well.

In this gum solution the pieces remain for twelve to forty-eight hours, according to their size and the time at the disposal of the investigator, and are then cut on one of the numerous ether-freezing microtomes now to be obtained



FIG. 17. SWIFT'S ETHER-FREEZING MICROTOME.

such as Swift's (fig. 17) or Cathcart's. A microtome in which the freezing is brought about by liquid carbonic acid is now being largely employed and acts well. The carbonic acid, contained in a cylinder, sprays on to the under surface of the plate on which the block of tissue rests; the tissue quickly freezes and is then cut. This form of microtome works well in the hottest weather. The material must not be frozen so hard that the sections roll up and fall off

the knife; the sugar in the above solution should prevent this. The sections are transferred successively to two or three lots of distilled water, preferably slightly warmed, to remove the gum, and can then be stained, or preserved in equal parts of methylated spirit and water until wanted.

Bacteria seem to retain their staining properties better in the tissue in bulk than when it has been cut up into sections. Although the bacteria may stain well in sections for some time after preparation, it frequently happens that in a month or two they refuse to stain. Such is certainly the case with anthrax tissues, but not with tubercle or leprosy, the bacilli in sections of the latter seeming to retain their staining properties unaffected for an unlimited time.

(b) *Paraffin Method.*—Nothing can surpass the paraffin method for the thinness and beauty of the sections obtainable by it, and for some friable tissues, such as actinomycosis, it is almost essential. The tissue, in suitable pieces for cutting, is transferred from the methylated spirit preservative solution to pure methylated spirit for two or three hours, and then to absolute alcohol—which may have to be changed once unless a fairly large volume is employed—for from four to twenty-four hours. It is then taken out, lightly compressed between the folds of a *dry* cloth or piece of blotting-paper to remove the superfluous alcohol, and placed in an excess of xylol. In this it remains for from four to twenty-four hours until cleared. This is recognized by the material assuming a more or less semi-transparent condition, and the process may be much accelerated by warming the xylol to from 37° to 50° C. in the blood-heat incubator or paraffin oven, the bottle containing the xylol being well stoppered. When cleared it is ready to go into the bath of melted paraffin. A paraffin of a fairly high melting-point is perhaps the best, viz. 45° to 55° C., and is placed in glass capsules in an oven which can be kept uniformly heated to the required temperature. An ordinary chemical hot-water

oven does admirably for this purpose, and is heated by a special form of small Bunsen burner with mica chimney, the temperature being regulated by some form of mercurial regulator which is set a degree or two above the melting-point of the paraffin employed. The tissue is taken out of the xylol, blotted to remove the excess, and placed in the melted paraffin for six to twenty hours. It is then embedded by pouring a little of the melted paraffin into a watch-glass, or into a small box formed of folded paper or lead-foil, or by bringing together two L-shaped pieces of brass on a glass plate so that a rectangular cavity is produced. The pieces of tissue are then taken out with a small warmed forceps or needle, adjusted to the position they are required to occupy, and more melted paraffin poured in, so as to cover them. When a film of solid paraffin has formed, the whole arrangement is carefully placed in cold water so as to cool it rapidly.

A new paraffin is frequently crystalline in structure, and acts much better after it has been kept melted for some weeks, or is much improved by heating nearly to its boiling-point for five or six days (P. T. Beale). The xylol for clearing may be used several times and the paraffin repeatedly, the remains of old tissues being removed. The time which the tissues require to remain in the alcohol, xylol, and paraffin depends upon their size; *very small* pieces may be treated in a few hours, large ones may require two or three days.

Other clearing agents, such as chloroform, turpentine, and cedar oil, may be used instead of xylol. The paraffin method is usually straightforward, but *small* pieces of tissue must not be left too long either in absolute alcohol or in the paraffin bath, for they are apt to become too hard to cut. Thyroid tissue and skin are also rather troublesome; they become very hard unless the whole process is carried out as rapidly as possible. If the pieces of tissue be large, the

capsule of melted paraffin containing the tissue may be placed under the receiver of an air-pump, which is then exhausted. This causes the paraffin to penetrate better, and the process may be repeated two or three times during the period of infiltration. A special form of paraffin oven has been devised by Cheattle for infiltrating under diminished pressure, and is made by Hearson, of Regent Street.

In order to prepare sections from material embedded in paraffin some form of microtome must be employed. An ether-freezing microtome *can* be employed with some

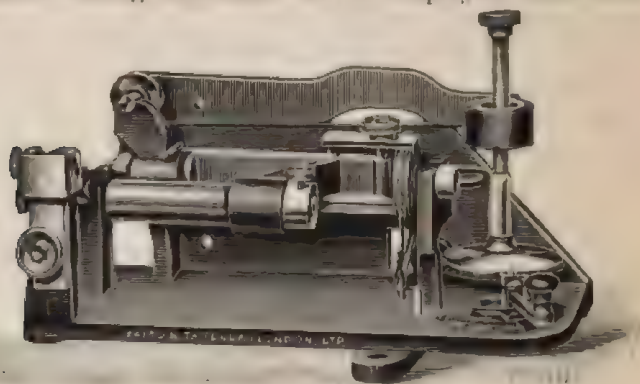


FIG. 18.—CAMBRIDGE ROCKING MICROTOME.

manipulation, the paraffin block being placed in a little melted paraffin on the freezing plate so that it is cemented there, and sections cut with the razor or plane iron, as though it had been frozen (it is *not* to be frozen). It is better, however, to use some special form of microtome, the Cambridge 'Rocker' (fig. 18), or a modification of it, or the Minot, being perhaps the best. The block of paraffin containing the tissue is trimmed with a knife to remove the excess, and is cemented to the carrier of the microtome with a little melted paraffin, or by melting the paraffin on

it with a hot iron (end of a file, &c.) or a match. The union may be made more secure by melting the paraffin around the base of the block with a hot iron.

Having fixed the paraffin block to the carrier, sections may then be cut of any degree of thinness. In order to do this it is essential for the knife or razor to have a keen edge and one of the right nature, for a knife may be perfectly sharp and yet the sections as they are cut roll up in such a manner that it is difficult to flatten them. Though this may be due to a wrong consistence of the paraffin, owing to cold weather or some other factor, in the majority of instances it is the edge of the knife which is at fault. Provided the knife be sharp, stropping on the palm of the hand will usually remedy this difficulty. The paraffin being of the right consistence, and the knife in good order, the sections as they are cut should be flat and should adhere together at adjacent margins so that a ribbon of greater or shorter length is formed.

Satisfactory sections having been obtained, they are transferred with a needle or camel's-hair brush to a tin pan containing a little water, or spirit and water, warmed to about 40° C. The sections float and the paraffin *softens*, so that they spread out perfectly flat (the water must not be hot enough to *melt* the paraffin). A clean slide is then introduced underneath the section, raised so that the section is lifted up on it, and by fixing the section with a needle and tilting the slide, the section is deposited in the required position on the slide and allowed to dry. If preferred, the section may be transferred to a slide flooded with water, which is warmed over the Bunsen. The slides can be manipulated in an hour or two if dried at 37° C., but it is best to allow them to dry in the incubator all night. It will be found after this treatment that the sections adhere sufficiently firmly to the slides for all the ordinary methods of staining to be carried out without

MANUAL OF BACTERIOLOGY

ing them, which would be fatal. The sections are fairly thin, however; if they are at all thick they do not adhere nearly so well.

If the sections have to be subjected to prolonged treatment during staining &c., they may be cemented to the slide by the following method. Equal parts of egg-white and cerin are mixed and filtered, and to every 100 c.c. of mixture 1 gram of sodium salicylate is added. The slide is covered thinly with this, covered with water, and the section laid in position. It is gently warmed until the water floats out flat; the water is then drained off, and the slide dried, after which it may be placed in a mixture of alcohol and ether for an hour.

Supposing that while cutting, the sections, in spite of the precautions, curl up instead of lying flat, it is still often possible to obtain a few that can be mounted. They may sometimes be unrolled by cautious manipulation with the point of needles after having been softened by warming.

It is necessary in the first place to have clean cover-glasses of the right kind: they must be thin, otherwise the higher powers cannot be employed to examine the preparations, and those described as 'No. 1' should be purchased, '¾-inch squares' being a convenient size. These serve both for cover-glass specimens and for covering sections; it is well also to have a few of the same thickness but larger, viz. ¾-inch or 1-inch squares, for large sections. In order to clean them they should be gently boiled in a porcelain dish with 10 per cent. carbonate of soda solution for a few minutes, well washed, and then treated with strong sulphuric acid, warmed carefully in a porcelain dish, for a few minutes. The acid having been poured off, they are well rinsed in several changes of water, and should be kept in a stoppered glass capsule in absolute alcohol. Slides may be used instead of cover-glasses, and should be cleaned and manipulated in the same manner as described for cover-glasses.

A clean cover-glass (or slide) is taken, dried with a clean soft linen or silk rag or handkerchief, or with Japanese paper, or it may be momentarily introduced into the Bunsen flame and the spirit burnt off, and placed flat on a convenient support on the work-table—a white glazed tile is excellent—with the corner projecting so that it can be conveniently picked up with the forceps.¹ A droplet (i.e. *small* drop) of water is then placed on it, in the middle, by means of a looped platinum needle, or with a small glass pipette (fig. 6). Theoretically, sterilized physiological salt solution² should be used, a few cubic centimetres being boiled in a sterilized and plugged test-

¹ The writer has devised a useful support for staining. It consists of a square of plate glass, painted half white and half black at the back, and having a narrow strip of thick glass cemented across it on which the covers rest. It is made by Messrs. Baird & Tatlock.

² 0.75-0.85 per cent. of sodium chloride dissolved in distilled water.

MANUAL OF BACTERIOLOGY

two or three minutes and cooled ; but ordinary
er may usually be employed. A thin film of
us has now to be formed on the cover-glass, and
wing is the method of procedure with a culture on
medium such as agar or gelatin. The culture tube
tinum needle are held and manipulated in precisely
e manner as that described for the inoculation of
(. 61), a trace of the growth being removed from the
l the wool-plug immediately replaced.

ere trace of the growth from a culture should be
ust sufficient to soil the tip of the platinum needle,
reparation will be too crowded, and this is rubbed
with the droplet of water on the cover-glass, so as
an emulsion, which is then spread over the surface.
neral rule the material should be well rubbed up,
ome instances this is inadvisable, as a particular
on or characteristic grouping might be disturbed
in which case, after a slight admixture with the

bottom having been shaken up if necessary, and a loopful or two of the fluid removed with a looped platinum needle, transferred to the cover-glass, spread out, dried, and fixed as before, but as the medium is fluid there is usually no need to add a droplet of water.

If a specimen of blood, pus, or sputum is required, the procedure is much the same. A little of the material is taken up with a looped platinum needle and spread in a thin film over the cover-glass or slide, which is then dried and fixed. If necessary, a droplet of water or physiological salt solution may be used to dilute the material so as to obtain a thinner film. If a specimen is to be made from an organ, a particle of the pulp is picked up and an emulsion made as before, or a small piece of the organ may be held in sterile forceps and the cut surface gently smeared over the cover-glass or slide, which is then dried and fixed; these are termed 'smear preparations.'

To obtain the best results it is preferable before staining to submit films of blood¹ or pus or smear preparations to the action of some chemical fixing agent, unless the film is stained with Leishman's solution, which both fixes and stains. The simplest method of doing this is to immerse the films, after *air*-drying, in a mixture of equal parts absolute alcohol and ether for five to fifteen minutes. In hot countries a saturated aqueous solution of corrosive sublimate (five to fifteen minutes) is perhaps as good as anything. Another method, combining both fixing and staining, is to immerse the films as soon as they are prepared and without drying in the following solution:

Absolute alcohol saturated with eosin	25 c.c.
Pure ether	25 c.c.
Alcoholic solution of corrosive sublimate (2 grams in 10 c.c.)	5 drops

¹ For the method of preparing blood-films see the section on Malaria.

MANUAL OF BACTERIOLOGY

Four cover-glasses 5 to 10 c.c. of this solution are placed in it, and they should remain in it three to four minutes (may be prolonged for hours without harm) ; they are then removed with a forceps and well rinsed in water, stained for less than a minute in a saturated aqueous solution of methylene blue, washed quickly, dehydrated in absolute alcohol, cleared in xylol, and mounted in xylol balsam. The same may be used for fixing blood, pus, sputum, &c., if fixation can be omitted, and the preparations may then be stained or otherwise treated in any desired manner.¹

² recommends the following as giving the most satisfactory results :

Hold the freshly prepared and still wet film in the mouth of a wide-mouthed bottle half filled with the formalin solution, film side downwards, for five minutes.

Drop, *while still wet*, film downwards into absolute alcohol. Leave for fifteen minutes, or, for convenience, for

from the tube. This can be done by dipping the tube for a few seconds into hot water; the gelatin round the walls of the tube will be melted, and the gelatin mass can then be tilted out of the tube on to a glass plate or tile.

STAINS AND STAINING METHODS.

Micro-organisms being so minute and transparent, it is usual to stain or dye them, so that they can be more readily examined. In some instances organisms may have a peculiar staining reaction, which may serve as an aid to their identification. But when an organism is being investigated, examination in the fresh and living condition must never be omitted, for it is only thus that its motility and life-history can be studied. Only general methods are detailed here; special ones will be given when they are required.

1. Löffler's Alkaline Methylene Blue.

Concentrated alcoholic solution of methylene blue . . .	80 c.c.
Solution of caustic potash, 0·01 per cent.	100 c.c.

A very useful staining solution. Cultures should be quite fresh, or the organisms do not stain well. When the organisms are mixed with extraneous material, as in smears, or there is much debris, this is one of the best staining solutions to employ. Methylene blue preparations are, however, not very permanent, and in hot countries rapidly fade. Thionine blue is then preferable.

Cover-glass specimens are stained for three to ten minutes, and sections half to twenty-four hours.

2. Carbol-Methylene Blue (Külme).

Methylene blue	1·5 gram
Absolute alcohol	10 c.c.
Five per cent. aqueous solution of carbolic acid . . .	100 c.c.

A more intense staining solution than the former, and very useful for sections, which are stained for from half to six hours.

MANUAL OF BACTERIOLOGY

Anilin Gentian Violet.

ed alcoholic solution of gentian violet	90 c.c.
water	100 c.c.

Anilin water is prepared by shaking 9 c.c. of anilin with distilled water, allowing the mixture to stand for a few days, and filtering. Instead of anilin water, 1 per cent. carbolic may be used (carbolic-gentian violet).

This solution is a useful general stain for films, which are stained for two or three minutes, and is employed in Gram's method of staining. It does not keep well.

Carbol-Fuchsin (Ziehl-Neelsen solution).

Fuchsin	1 part
absolute alcohol	10 parts
5 per cent. aqueous solution of carbolic acid	100 parts

Fuchsin is dissolved in the absolute alcohol and then mixed with the carbolic acid solution. It must always be used before use.

This is an intense staining solution. For cover-glass specimens

Film specimens, after fixing, are placed in absolute alcohol for half a minute, dried, and placed, film downwards, in a watch-glass of the stain, which is then warmed until it steams. The specimen is then taken out and well washed in tap-water, rinsed in distilled water, dried, and mounted.

Sections are immersed in absolute alcohol for several minutes, stained in the cold for six to twelve hours, well washed in distilled water, and passed through absolute alcohol and xylol, and mounted.

7. Eosin (alcohol-soluble and water-soluble).

A somewhat diffuse stain. Is used for counter-staining the tissues in Gram's method, and for staining blood-corpuscles and leucocytes.

A $\frac{1}{2}$ to 1 per cent. aqueous or alcoholic solution should be used, and the staining should not, as a rule, be prolonged for more than about half a minute.

8. Bismarck Brown.

A saturated aqueous solution should be prepared and diluted somewhat for use. A good counter-stain for the tissues in Gram's method. Stain for two to five minutes.

9. Orange-rubin.

Prepare saturated aqueous solutions of Orange G. and Rubin S. Mix equal volumes and dilute with water until of a light port-wine colour. Stain tissues for five to fifteen minutes. A good contrast stain for tuberculosis and actinomycosis.

10. Picro-carmin.

This is best bought ready prepared. Sections are stained in the solution for half to one hour, washed, then placed in a watch-glass of spirit, to which three or four drops of hydrochloric acid have been added, for two or three minutes, then well washed in water. The section can now be counter-stained with Löffler's blue or by Gram's method.

MANUAL OF BACTERIOLOGY

Hæmatoxylin.

h's formula is one of the best and simplest to use, and obtained ready for use. It must be 'ripe.' It is a al and not a bacterial stain. Sections are treated as

distilled water, one to two minutes.

ain with the hæmatoxylin solution for five to thirty

In some cases the solution is preferably diluted some-
distilled water.

use in distilled water.

use in distilled water containing a *trace* of acetic acid.

eat with distilled water containing a *trace* of ammonia.

ons remain in this until they assume a deep blue colour.

er, five to ten minutes, may also be used.)

ey can then be dehydrated, cleared and mounted, or

ained with eosin, orange-rubin, or Van-Gieson, and
nted.

toxylin makes a good contrast stain for the tubercle
prosy bacillus and for *Actinomyces*.

14. Giemsa Stain.

An eosin-azur mixture dissolved in glycerin and methyl alcohol. Useful for blood-films, film preparations, &c., and has been much used to demonstrate the spirochaetes in syphilitic material. (For method of using, see 'Syphilis'.)

15. Beale's Carmine.

Carmine (best)	10 grains
Liq. Ammon. Fcrt.	$\frac{1}{2}$ drachm
Pure glycerin	2 ounces
Alcohol	$\frac{1}{2}$ ounce
Distilled water	2 ounces

Used for staining *fresh* tissues and protozoa.

Safranin and acid fuchsin are also used as counter-stains. Malachite green, neutral red, and rosein may be used for *intra-vitum* staining of protozoa &c.

Eosin, orange-rubin, hæmatoxylin, picro-carmine, and Beale's carmine keep well in solution; the remainder may or may not, and are best used fairly fresh. All stains should be filtered before use, and may be conveniently kept in bottles having a funnel fitted with a filter-paper so that they are always ready. Or smaller bottles may be used, fitted with pipettes, and several arranged in a stand.

The best stains are Grübler's, which can be obtained from many agents in this country. Messrs. Burroughs, Wellcome & Co. supply most of the anilin dyes and some other reagents, iodine, &c., in 'soloids,' which are very convenient and good.

Gram's Method.—This is a most useful method, especially for sections, specimens of blood, or smear or impression preparations, as the tissue or ground substance can be counter-stained so that the organisms show up in marked contrast. Ordinary cover-glass specimens do not usually require this method, unless debris or ground substance is present and the best result is desired. Unfortunately Gram's method is not applicable for all organisms, as many do not retain their colour by the process. This

MANUAL OF BACTERIOLOGY

stage, however, is counterbalanced by the fact that it is a valuable means of distinguishing organisms, and is one of the points to be noted in bacteriological work. Most of the moulds, yeasts, streptothrix and other filamentous forms, and cocci stain by it, though there are exceptions; the spirilla and protozoa do not stain by it, and regards the bacilli no rule can be laid down (see p. 100). Cover-glass specimens are stained for five to ten minutes and sections for ten minutes to half an hour, in a 1 per cent. carbol-gentian violet solution. Drain off the excess stain, and then immerse, without washing, in a 1 per cent. fast green solution for one half to two minutes:

Carboline	1 part
Potassium iodide	2 parts
Distilled water	300 parts

The purple colour of the gentian violet changes to a dirty brown, and sections become much like a used

longer than thin ones. It is also important with cover-glass specimens to remember on which side of the glass the film is, for it may be very difficult to ascertain this when the specimen has been decolorized. After decolorizing, cover-glass specimens are washed in water, dried, and mounted, or, after washing, the ground substance may be counter-stained, if required, with eosin for a few seconds, or Bismarck brown for two or three minutes, washed again in water, dried, and mounted. Sections after decolorizing are passed through absolute alcohol and xylol before mounting, or, if required to be counter-stained, are immersed in eosin for fifteen to thirty seconds, or Bismarck brown for three to five minutes, and then passed through methylated spirit, absolute alcohol, and xylol.

Sections frequently are somewhat difficult to decolorize with alcohol alone, in which case it is well to treat them with a slightly acid alcohol (3 per cent. of hydrochloric acid) for a few seconds, and then return to the alcohol (Gunther's method).

The iodine in Gram's method seems to act as a mordant, precipitating the stain in a relatively insoluble form in certain species of bacteria. The staining of organisms by Gram is relative; some forms do not stain at all, are *Gram-negative* i.e. the colour is removed by the alcohol with the greatest facility—others stain intensely, are *Gram-positive*, but even these may become decolorized by prolonged treatment with alcohol. The best procedure is to have three watch-glasses of methylated spirit, and to transfer the cover-glass specimen from one to another as the stain is dissolved out; in the last bath, immediately the final trace of colour is seen to be dissolved out, the preparation should be removed, washed in water, dried, and mounted. In order to ascertain whether an organism is or is not stained by Gram's method it is sometimes useful to mix with it in making the preparation some

MANUAL OF BACTERIOLOGY

ed Gram-staining organism—e.g. if a bacillus, the *Micrococcus pyogenes*; if a coccus, with *racis* or *B. subtilis*. The admixed organism then is an index.

Following organisms are Gram-positive: *B. anthracis*, *B. tetani*, *B. Welchii*, *B. botulinus*, *B. tuberculosis*, *B. lepræ*, *B. murisepticus*, *Actinomyces*, *B. subtilis*, *B. megaterium*, *B. mycoides*, the pyogenic cocci, staphylococci, including the pneumococcus, most cocci, yeasts, and streptothrices.

Following organisms are Gram-negative: *B. typhosus*, *B. dysentericæ*, *B. coli*, *B. pestis*, *B. influenza*, *B. pseudotuberculosis*, *B. pyocyaneus*, *B. oedematis*, *B. Chauvæi* (usually), *B. prodigiosus*, *B. proteus*, the pathogenic bacilli such as chicken cholera, the spirilla and spirochaetes and protozoa, *M. gonorrhœa*, *M. meningitidis*, *M. melitensis*, and *M. catarrhalis*.

W. H. Audius's modification of Gram's method, staining

removed with filter-paper and the sections are flooded with anilin oil two or three times. This removes the colour and dehydrates. The anilin oil is removed by flooding two or three times with xylol.

Thionine blue may be used for Gram's method, the carbol solution being employed (No. 5, p. 90). Sections are stained for two or three minutes, then treated with an iodine solution somewhat stronger than Gram's (200 parts of water instead of 300 parts). The sections, after remaining in this for one to two minutes, are decolorized in alcohol containing 1 per cent. of acetone (methylated spirit does very well), and subsequently treated as in Gram's method.

THE STAINING OF FILM SPECIMENS.

To stain cover-glass, smear, and impression preparations, after fixing, the film is flooded with a drop or two of the solution, or the preparation is floated, film side down, on the solution contained in a watch-glass; if it should sink it makes little difference. Various baths or pots can be obtained for staining slides. Warming intensifies the staining properties of all staining solutions. Having stained sufficiently, the preparation is rinsed in a beaker or tumbler of water, or in a fine stream of water, preferably distilled, and having rinsed the superfluous colour away, it is dried and mounted on a glass slide, film side down, in a drop of Canada balsam dissolved in xylol. The preparation is dried by *gentle* warming over the Bunsen flame, having first drained it on to a piece of filter-paper for a second or two, remaining droplets of water being mopped up with a shred of filter-paper, or the film may be blotted between filter-paper. Or the film may be allowed to dry spontaneously in the air, in which case it should always be set up on edge to drain, preferably on a ledge of filter-paper, which is folded into a sort of

MANUAL OF BACTERIOLOGY

sed Z (z) shape. *The preparations must be
ly dried before being mounted in balsam.*

ere be much debris or other material which, when
would interfere with a clear view of the organisms,
expedients may be adopted. One is to stain for a
ne with a solution which does not give a very dense
ne best for this purpose being Löffler's methylene

Gram's method may be made use of if the
stains by it, and will give the best result of any.

plan is to treat the specimen with acetic acid
aining; it may be just dipped in glacial acetic acid
mediately washed in distilled water, or immersed
er cent. acetic acid for five to ten minutes, washed
led water, and then stained. A third is, after

and washing, to rinse the preparation in dilute
methyiated spirit 1 part, water 1 or 2 parts), and
tely wash again in water to stop the further action
cohol. If the film be thick, two or three rinses

TREATMENT OF SECTIONS FOR STAINING AND MOUNTING.

(a) *Frozen Sections*.—If preserved in spirit they should be rinsed in distilled water before staining, unless the staining solution is an alcoholic one, in which case this is unnecessary. After staining they are well rinsed in water or methylated spirit to remove the excess of stain, and then dehydrated and cleared before being mounted. For dehydrating, if they have been washed in water, they should be well rinsed in methylated spirit¹ to remove the excess of water, and then transferred to absolute alcohol for a few seconds to two minutes, the time varying with the size and thickness of the section. In many cases—for instance, when the anilin dyes have been used for staining—the sections must be passed as rapidly as possible *consistent with thorough dehydration* through the absolute alcohol to avoid removing too much of the colour. If it is important to avoid any decolorization, anilin oil may be used for dehydration, as in Weigert's method (pp. 96 and 102). For clearing, xylol or cedar oil is the best agent, for neither dissolves the anilin dyes; they will only clear, however, out of absolute alcohol: hence the preliminary rinsing of water-washed sections with methylated spirit to prevent dilution of the subsequent bath of absolute alcohol. Oil of cloves can also be employed, but has the disadvantage that it dissolves the anilin dyes, and the colour of stained sections treated with it is apt to be less permanent; it has the advantage, however, of clearing out of methylated spirit, absolute alcohol being unnecessary. The alcohol and clearing agents are conveniently placed in watch-glasses or small shallow glass capsules. The section is known to be cleared when it appears quite transparent and almost

¹ Absolute alcohol may of course be employed instead of the first bath of methylated (or rectified) spirit, but methylated answers just as well and is less expensive.

MANUAL OF BACTERIOLOGY

when the watch-glass or capsule containing it is over a dark surface. If it appears cloudy and opaque it is not properly cleared, which results from insufficient clearing or dehydrating. If the section does not clear in a day or two it is evidently not sufficiently dehydrated, and should be returned to a fresh bath of absolute alcohol for a short time, and then transferred again to the clearing agent. Care should be taken that watch-glasses, &c., used for the absolute alcohol and clearing agent are perfectly dry. The clearing agent, especially clove oil, can be used many times before becoming useless.

On transferring the sections from one solution to another, an ordinary needle, fixed in a light wooden handle, or, better still, a piece of glass drawn out at one end, the section being carefully lifted by one corner to prevent wrinkling; but for the final process of mounting it is better to use a section lifter or cigarette-paper. The paper, spread out with care, is raised up by means of the

immerse in (1) xylol for one to two minutes; (2) absolute alcohol one to two minutes, to remove the xylol; (3) methylated spirit; (4) distilled water. They are now ready for staining, and are to be flooded with the staining solution or immersed in it, and after staining they are treated in the same manner, but in the reverse order, i.e. (1) distilled water; (2) methylated spirit; (3) absolute alcohol; (4) xylol. On being removed from the xylol the slides are drained for a few seconds, a drop of xylol balsam is then put on, and the section covered with a clean cover-glass. Glass pots (fig. 19) filled with the alcohol, xylol, &c. are convenient for the treatment of paraffin sections, the slide with the section upon it being immersed in the fluid.



FIG. 19. GLASS POT FOR CLEARING ETC.

SECTION STAINING.

When Gram's method is applicable it gives by far the best results, and should always be employed. If, however, the organisms are decolorized in Gram's process, some other method must be adopted. One of the best is to stain for from ten minutes to six or eight hours in Löffler's methylene blue. Fresh easily staining organisms will be sufficiently stained in ten or fifteen minutes, but when the organism is difficult to stain, as glanders, six to eight hours may not be too long a time. Warming intensifies the staining properties of all staining solutions; for frozen sections the watch-glass of stain may be warmed on a sand-bath or asbestos cardboard, or in the blood-heat incubator. Sections on the slide may be flooded with the stain and warmed on a piece of asbestos cardboard placed over a Bunsen flame. After staining, the sections are well rinsed

MANUAL OF BACTERIOLOGY

al water and then slightly decolorized by rinsing a minute or so in a watch-glass of 1 per cent. in distilled water. They are then again washed as rapidly as possible through alcohol, cleared and mounted. Carbol-methylene blue or carbol-blue may be used instead of the Löffler's solution, staining taking from a few minutes to half an hour. If a fast stain be desired, the sections may be treated a few seconds with the eosin solution after the dilute stain. A better method is to use the Chenzinski solution which is strongly recommended by Klein (see p. 90). If the staining be prolonged, evaporation must be prevented. If a section mounted on the slide and flooded with water, the slide should be placed on a piece of wet paper on a tile and covered with the lid of a Petri

micro-organisms in sections stained with Löffler's solution are very liable to become decolorized unless the decolorization is expeditiously performed. To avoid this Hanna's

be as thick as the bacterial cell itself, and appears, in the unstained state or after staining by the ordinary methods, as a clear halo or zone surrounding the organism. Organisms in films of albuminous matter often appear to be surrounded by a clear halo which must not be mistaken for a capsule. As organisms frequently lose their capsules on ordinary culture media, Moore recommends cultivating in fluid serum to obtain the re-development of the capsule. In order to stain the capsule, one of the following methods may be adopted:

1. Stain the preparations by just dipping in the following solution:

Carbol-fuchsin	1 part
Distilled water	1 part

Rinse in water and then stain for fifteen seconds in a very weak aqueous solution of gentian violet (0.1 per cent.) Rinse in water, dry, and mount.

2. *McConkey's Method.* The following solution is prepared:

Methyl green	1.5 grains
Dahlia	0.5 gram
Distilled water	100 c.c.

When dissolved, 10 c.c. of a saturated alcoholic solution of fuchsin are added, and the whole is made up to 200 c.c. with distilled water. The stain should not be used for a fortnight, and should be kept in a dark place. Specimens are stained for five minutes or longer, and thoroughly washed in a stream of water, dried, and mounted.

3. *Friedländer's Method* (for tissues).—Mix,

Concentrated alcoholic solution of	
gentian violet	50 parts
Distilled water	100 parts
Acetic acid	10 parts

Stain the sections in this solution in the warm incubator for twenty-four hours. Rinse well in 1 per cent. acetic acid, pass through alcohol and xylol, and mount in balsam.

MANUAL OF BACTERIOLOGY

SPORE STAINING.

When spore-bearing bacteria are stained by the ordinary method, the spores are just tinted, or remain uncoloured, while the outlines more or less stained. This seems to be due to the fact that the spores are surrounded with a tough membrane which prevents the entrance of the staining agent. By staining by some method which causes the penetration of the stain and then cautiously decolorizing, it is possible to remove the colour from everything except the spores, the resist membrane of which in the same way prevents the full action of the decolorizing agent.

Simple Method.—A film is prepared in the ordinary way. If on cover-glass, it is floated on a watch-glass of, if a slide, it is stained with carbol-fuchsin, and the stain is warmed for twenty minutes. After being washed in water, the preparation is rinsed with alcohol and or two in 1 per cent. sulphuric acid and again washed in water. If there is still a good deal of the red colour

specimen in the ordinary way. Treat with absolute alcohol for two minutes, and then with chloroform for two minutes. Wash in water and treat with a 5 per cent. solution of chromic acid for two minutes, wash, and then stain with warm carbol-fuchsin for ten minutes. Wash, decolorize carefully in 1 per cent. sulphuric acid, again wash, and counter-stain with Löffler's methylene blue for one minute, wash, dry, and mount. Some organisms, such as the *B. mesentericus*, stain better if treated with the chromic acid for five to ten minutes.

FLAGELLA STAINING.

Many organisms possess delicate protoplasmic processes, flagella, in greater or less number; but these are not visible when the organism is examined in the living condition (except by the use of dark ground illumination), nor when the ordinary staining methods are employed. In order to demonstrate them it is necessary to make use of some special method, in which a mordant is essential. One of the earliest devised was that of Löffler, which with care gave fair results. It is not, however, nearly so satisfactory as some more recent ones, so is omitted.

For all methods of flagella staining the cover-glasses or slides must be absolutely clean, the cultures recent, and the growth sufficiently diluted to obtain the organisms in an isolated condition.

(a) *Stephens's Method.*

This is a modification of the well-known Van Ermengem method,¹ and has been communicated to the writer by Dr. J. W. W. Stephens.

To clean Slides.—Rub the slides with a clean cloth and place on a piece of clean wire gauze and heat with a *smokeless* flame for some minutes (by this means grease is completely removed). Remove the slides when cool, not before.

To make the Emulsion.—All methods are unsatisfactory. Rub a little of the culture in a small drop of tap-water in a

¹ *Centr. f. Bakt.* xv., 1894, p. 969.

MANUAL OF BACTERIOLOGY

ss. Then transfer a drop with the smallest possible loop to a minute drop of water on the slide. Mix and with the platinum wire as quickly as possible. The film should dry *immediately* if a small drop only of water used.

of the Culture.—A twenty-four hours' culture does quite younger one is perhaps better, but flagella can be shown (week or fortnight or more).

The Mordant :—

Osmic acid, 2 per cent.	1 part
Tannic acid, 20 per cent. watery solution		8 or 4 parts

*Silver Solution :—*Silver nitrate 1 per cent.

Gallic acid, 2 per cent. solution 1 part

Ammonia fort. 1 part

be mixed before using, and to be used immediately.

Stain.—Place the mordant on the film for one or two or less (time unimportant).

Wash in tap-water thoroughly.

Wipe off as much water as possible.

(b) *Pittfield's Method*.—Two solutions are freshly prepared :

- | | |
|--|---------|
| A. Saturated aqueous solution of alum | 10 c.c. |
| Saturated alcoholic solution of gentian violet | 1 c.c. |
| B. Tannic acid | 1 gram |
| Distilled water | 10 c.c. |

The solutions should be made with cold water, filtered, and preserved in separate bottles. For use equal quantities are mixed together. The specimens are flooded with the mixture and held over the flame until it nearly boils; they are then laid aside, with the hot stain on them, for one minute, and are finally washed in water. After washing, the preparations are flooded with anilin gentian violet for one second, washed in water, dried, and mounted.

(c) *McCrorie's Method*¹ (modified by *Morton*²).—Prepare the following solutions :

- | | |
|------------------|----------|
| A. Tannic acid | 1 gram |
| Potash alum | 1 gram |
| Distilled water | 40 c.c. |
| B. 'Night' blue | 0.5 gram |
| Absolute alcohol | 20 c.c. |

Mix and filter.

The prepared slides are stained with this solution (which should always be filtered before use) for two minutes, the solution being changed two or three times, washed gently in running water, and then counter-stained in anilin gentian violet for one to two minutes, washed, dried, and mounted.

PRESERVATION OF CULTURES.

Gelatin and agar cultures may be satisfactorily preserved by submitting them to the action of formaldehyde vapour for some hours by soaking the wool plug of the culture tube in formalin and plugging the tube with it. The tube may then be sealed with gutta-percha tissue, sealing-wax, or paraffin wax, or best of all in the blowpipe flame. Plate cultivations may also be exposed to the vapour and the lid of the dish afterwards

¹ *Brit. Med. Journ.* 1897, i. p. 971.

² *Trans. Jenner Inst. Prev. Med.* ii. p. 242.

MANUAL OF BACTERIOLOGY

ed on, or the cultures may be made in the flat bottles (see p. 101) devised for the purpose, and after development treated like tube cultures.

PRESERVATION OF PATHOLOGICAL SPECIMENS.

These may be preserved in the ordinary way in spirit, but a better method, by which the natural colour of the specimen is retained, is the following. The specimens are first placed in water, and then placed in the following solution for four to forty-eight hours:

Formalin	6 parts
Sodium chloride	1 part
Sodium sulphate	2 parts
Magnesium sulphate	2 parts
Tap-water	100 parts

After being taken from the formalin solution the specimens are placed in methylated spirit for ten minutes, and then in a bath of methylated ; in this the colour to a large extent is retained, and they should be carefully watched and not allowed to

CHAPTER IV.

METHODS OF INVESTIGATING MICROBIAL DISEASES THE IN-
OCULATION AND DISSECTION OF ANIMALS —HANGING-DROP
CULTIVATION—INTERLAMELLAR FILMS—THE MICROSCOPE.

The systematic study of conditions dependent on the activity of micro-organisms is in many instances no light matter. When only one or two forms are present and these are readily cultivated it may be comparatively easy, but when there are many species the matter may become exceedingly complicated. The first step to be taken is to ascertain what organisms are present in the material and their distribution by careful microscopical examination, both in the fresh condition and in stained preparations, and if possible at different stages of the disease. In disease conditions, for example, the blood and secretions may be examined both before and after death, but in the latter it must be remembered that soon after the fatal event adventitious organisms rapidly make their appearance, gaining access from the air and from the intestinal tract. If organisms are detected an attempt should be made to determine if there is any predominant form and if this is constantly present at different stages. If organisms are found, it simplifies matters; but if not, it cannot therefore be said that they are absent, for they may be few in number, and consequently be missed in a microscopical examination; or they may be confined to a particular locality or tissue, or are present only at one stage of the

MANUAL OF BACTERIOLOGY

8. In addition to the microscopical examination, must be made on various media, those media being which will probably be suited to the growth of the present in the particular condition ; for example, in the diseases of animals, media rich in protein, blood serum, peptone-agar, and gelatin, will be the serviceable. In examining the bacterial diseases of infusions prepared from the plant itself, and enriched by the addition of vegetable proteins, and carbohydrates, should be chosen. In fermentations, beer-wort, or fruit juice, and saccharine solutions should be used ; while for the nitrifying organisms, solutions containing nitrates and nitrites, salts of ammonia, urea, and agarin will have to be employed. In addition, it is in most cases be advisable, and in all safer, to make serial dilutions, in order to isolate the various species, and obtain pure cultures. Having obtained pure cultures, it will be necessary to determine the species of

3. The peculiarities of staining, and the staining reaction with Gram's and the Ziehl-Neelsen method.

4. The characters of the colonies in gelatin, agar, and other media, both surface and deep.

5. The characters of the growth on a variety of culture media at different temperatures—for example, for a pathogenic organism on blood serum, agar, and gelatin (surface and stab cultures), in broth and on potato; liquefaction or not of the gelatin; the growth in milk, with or without curdling, and the reaction therein; and the fermentation reactions on carbohydrates, glucosides, alcohols, &c.; the nature of the gas, if any, formed therefrom, and the $H : CO_2$ ratio.

6. The behaviour towards oxygen—is it aerobic or anaerobic?

7. The range of growth at different temperatures.

8. The reducing power by growing in litmus broth which becomes decolorized, or by the formation of nitrites in a solution containing nitrates.

9. The production of indole with or without nitrites.

10. The production of pigment and the conditions under which it occurs.

11. The pathogenic action on various animals if it be a disease germ, or the changes which it produces if it be an organism connected with other conditions.

12. The chemical changes which it induces.

13. The thermal death-point and the action of germicides and antiseptics upon it (see Chapter XX.)

For descriptive purposes, 'standard' culture media should always be employed, and the acidity or alkalinity of the medium stated (p. 56).

It must never be forgotten that under cultivation the properties of organisms may be considerably modified, and due allowance must be made for this. For example, pathogenic organisms may lose their virulence more or less

MANUAL OF BACTERIOLOGY

ely, pigment production be lost, and fermentive modified. An instance of the latter is given by Percy and ; a bacillus isolated by him possessed the power fermenting calcium glycerate, but after cultivation on y gelatin it completely failed to do so.

obviate these difficulties the organisms should be ed under as nearly natural conditions as possible -cultivation avoided as far as can be. No general be given as to the duration of life of cultures on media. Most organisms will retain their vitality ast three or four weeks without being transferred h soil, some for many months ; a few must be sub- every week, or they will die out ; while there are mall number, such as leprosy and relapsing fever, o far have rarely or never been cultivated. On the organisms retain their vitality best on gelatin.

an organism to retain its virulence it is, as a rule, y to pass it through a susceptible animal at longer

The slightly virulent organism will then be able to grow under the more favourable conditions, and a form which has become completely non-virulent can be made to regain its lost virulence.

Collodion sacks are now frequently used to study the action upon animals of the dialysable products produced by micro-organisms which do not form any appreciable amount of toxin *in vitro*, for cultivating species which are difficult to grow by ordinary methods, for studying the phenomena of infection when the micro-organisms are protected from the phagocytes, and for other purposes. A glass rod or small test-tube, according to the size desired, is dipped into a beaker containing the ordinary (*not* flexible) collodion, is then withdrawn and allowed to dry, and the process repeated two or three times. In order to detach the collodion from the glass, the whole is dipped for a few seconds alternately into strong spirit and into water, the collodion loosens, and may be easily peeled off the glass. The sack may be sterilized by placing in a test-tube and heating to 150° C. in the hot-air sterilizer.

For the inoculation of animals various methods may be adopted. In some cases, after clipping the hair, the organism may be introduced by rubbing into the skin after scarification, or, a small incision having been made through the skin, a small quantity of a culture may be introduced on a platinum needle; or a broth culture or an emulsion, made with sterilized water or broth, may be injected with a sterilized syringe subcutaneously, intraperitoneally, or into the muscular or other tissues or organs as required, since the seat of inoculation may have to be varied for the different species to produce their pathogenic effect. For injection purposes a syringe like an antitoxin syringe, i.e. with asbestos piston and glass barrel that can be boiled, should be used. Several sizes are kept in stock; 1 c.c., 2 c.c., and 5 c.c. at least being required. An all-

glass syringe is a still better form, but is expensive. For accurate dosage, the piston-rod should be graduated and have a nut travelling on a screw up and down it. Before use the syringe with the needle should be boiled for ten minutes to sterilize it; after use it may be well rinsed and again boiled. The needles should be wiped dry and a wire inserted, or they may be kept in a bottle of xylol.

Guinea-pigs and rabbits are usually inoculated in the thigh or abdomen; mice in the dorsal region or at the root of the tail (dorsally), the hair being clipped, and the skin disinfected, but this is not generally necessary. Numerous mechanical holders have been devised for animals, but are not as a rule required. Rabbits may be inoculated intravenously by one of the large veins in the ear. The hair is clipped, and the skin is well washed with a little alcohol with vigorous rubbing; the base of the ear is lightly pinched so as to obstruct the venous but not the arterial circulation, and render the vein prominent, and the injection made with a small syringe fitted with a fine needle, the needle being passed into the vein towards the base of the ear. After the withdrawal of the needle the wound is compressed for a little and may be dressed with some antiseptic wool and collodion.

The phenomena occurring after inoculation must be noted. Usually these are not very obvious in the rodents, but loss of appetite, sluggishness, staring coat, convulsions, &c. may be seen. The weight of the animal is a good index of what is happening. If the infection is serious, the weight rapidly falls; if the animal is to recover, its weight soon begins to increase after the preliminary fall. The temperature in the rectum may also be taken, but is not so valuable, as in the guinea-pig variations occur from mere handling or other slight causes. The temperature of the guinea-pig averages 38.6°, but varies between 36° and 39° C. (Eyre).

The examination of the dead animal should be carried out with as little delay as possible. For dissection, the body should be pinned out on the back on a board, which may stand in a shallow enamelled iron pan, by pins or nails through the feet, and the abdomen well soaked with antiseptic solution, not so much to sterilize the skin as to prevent the hair from getting into the incision; to obtain complete sterilization of the skin, it is preferable to clip or shave the hair and then sear with a red-hot iron. Knives, forceps, scissors, &c. should be well boiled in an enamelled-iron mug or pie-dish, the water being kept boiling during the progress of the dissection and the instruments rinsed from time to time in it. A little sodium carbonate may with advantage be added to the water. A small enamelled-iron fish-kettle with perforated strainer forms an excellent sterilizer for instruments, or a surgical instrument sterilizer may be used. An incision is made and the skin well reflected and pinned out; the knife and forceps should then be re-sterilized, or fresh sterile instruments taken, for the deeper incision and opening the body cavities; these again must be re-sterilized, or a third set of instruments employed for incising the organs.

During the progress of the dissection the condition of the tissues at the seat of the inoculation should be noted, and likewise the conditions of the serous membranes and the various organs. In many diseases the organism is met with most abundantly in the spleen, in others in the blood, and in some at the seat of inoculation. Where a systematic examination is made, film specimens and cultures on two or three media, aerobic and anaerobic, should be prepared from the seat of inoculation, the spleen, liver, lungs, and heart-blood, and in some cases from the serous membranes, muscles, or central nervous system in addition, the carcase being in the intervals covered with a bell-jar which has been rinsed in, or with filter-paper moistened with,

MANUAL OF BACTERIOLOGY

ic solution. An assistant is often useful or even necessary. The greatest care must be taken to avoid dropping or otherwise disseminating infective material, and the hands being immediately swabbed up with antiseptic solution; and the operator must exercise every precaution to prevent the infection of himself and others. It is convenient to have some efficient antiseptic solution near at hand which may be kept in a large bottle on a wall bracket and drawn off as required by a syphon tube provided with a tapping clip. The most generally used antiseptics are 1 per cent. carbolic, and 1-500 corrosive sublimate, but 2 per cent. formalin or kerol, or 3 per cent. lysol is cheaper and more efficient. The access of flies to the carcase must also be guarded against, as they might carry infection. When the dissection is over, with, the carcase should be efficiently disinfected and disposed of without delay, preferably by burning it, together with the board on which it has been pinned out.

passes superficially across the inner aspect of the thigh ; this permits of the withdrawal of a small quantity of blood without the necessity of an operation such as is required to expose the carotid. The simplest method of all is to introduce the fine point of a piece of glass tubing, drawn out and bent to a convenient angle, into one of the ear veins and aspirate the blood into it.

Blood may be obtained from a patient for the agglutination reaction, for microscopical examination, or for culture experiments, by pricking the finger or the lobe of the ear with a sterile needle, preferably a flat one of the 'Hagedorn' type, or with half a steel pen (nib) or a glass point; for disinfection, the skin may be rubbed with a little alcohol or ether. The blood may be collected in vaccine tubes, small bulbous tubes (fig. 6, p. 44), or Wright's tubes (fig. 34, p. 193).

Organisms, in natural infections in man, are usually present only in small numbers in the blood, and for demonstrating them by culture methods it is necessary to withdraw 2-5 c.c. from a superficial vein by means of a sterile syringe under aseptic conditions, and to inseminate broth tubes or agar plates each with $\frac{1}{2}$ c.c. of the blood.

Although the modern methods of isolation and cultivation have rendered immense service to bacteriology, they have also had the effect of diminishing the attention paid to the exact morphology and biology of organisms. At the present time there is a tendency to investigate bacteria *en masse* rather than to study them as individual living forms, and the following remarks by the late Marshall Ward¹ may be aptly quoted in this connexion:

'We must remember that De Bary and Brefeld had aimed at obtaining a single spore isolated under the microscope, and tracing its behaviour from germination continuously to the production of spores again; and when we learn how serious were the errors into which the earlier investigators of the mould fungi

¹ *Nature*, lvi., 1897, p. 455 *et seq.*

and yeasts fell, owing to their failure to trace the development continuously, from spore to spore, and the triumphs obtained afterwards by the methods of pure cultures, it is not difficult to see how inconclusive and dangerous all inferences as to the morphology of such minute organisms as bacteria must be unless the plant has been so observed. As a matter of fact, the introduction and gradual specialisation of Koch's methods of rapid isolation of colonies encouraged the very dangers they were primarily intended to avoid. It was soon discovered that pure cultures could be obtained so readily that the characteristic differences of the colonies in the mass could presumably be made use of for diagnostic purposes, and a school of bacteriologists arose who no longer thought it necessary to patiently follow the behaviour of the single spore or bacillus under the microscope, but regarded it as sufficient to describe the form, colour, markings, and physiological changes of the bacterial colonies themselves on and in different media, and were content to remove specimens occasionally, dry and stain them, and describe their forms and sizes as they appeared under these conditions. To the botanist, and from the point of view of scientific morphology, this mode of procedure may be compared to what would happen if we were to frame our notions of species of oak or beech according to their behaviour in pure forests, or of a grass or clover according to the appearance of the fields and prairies composed more or less entirely of it, or—and this is a more apt comparison, because we can obtain colonies as pure as those of the bacteriologist—of a mould fungus according to the shape, size, and colour, &c. of the patches which grow on bread, jam, gelatine, and so forth.'

EXAMINATION IN THE FRESH STATE.

One essential procedure in the investigation of an organism is its examination in the fresh and living condition. This may be done by placing a droplet of sterile water, broth, or salt solution on the slide, inoculating with a trace of the organism or growth, and covering with a cover-glass and examining microscopically. The action of stains and reagents on the organisms may be observed by

the irrigation method. A drop of the stain or reagent (c, fig. 20) is placed on the slide A, just in contact with one margin of the cover-glass, B, and is drawn through the preparation by means of a small piece of filter-paper, D, placed on the other side, a torn margin touching the film of fluid at one edge of the cover-glass.

The filter-paper absorbs the fluid from under the cover-glass, leaving the cells and other particles behind, and at the same time the reagent on the opposite side flows under the cover-glass to take the place of the absorbed fluid. Afterwards the excess of the reagent or stain may be washed away by running in water under the cover-glass

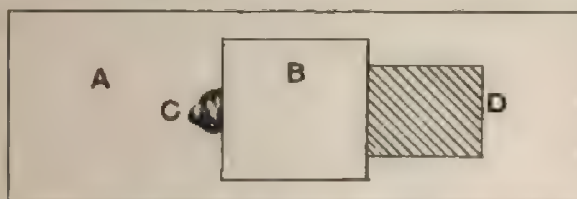


FIG. 20.—METHOD OF IRRIGATION.

in a like manner. Care must be taken that no fluid gets on to the upper surface of the cover-glass, which must always be kept dry. The advantage of this method is that it may be applied while the specimen is being examined under the microscope, and the action of the reagent on a particular cell or granule can, with a little care, be watched. If the cells be large and it is desirable to avoid pressure of the cover-glass, a fine hair or bristle may be so placed on the slide that when the cover-glass is lowered one edge rests on it. If the specimen has to be kept for any length of time, the film of fluid will before long evaporate and become dry. To prevent this a ring of oil or vaseline may be painted round the margin of the cover-glass so as to seal it to the slide.

MANUAL OF BACTERIOLOGY

simple method for keeping organisms under examination for a lengthened period of time, and of watching growth and development, is by the use of hanging-drops. A hollow-ground slide is sterilized by passing six times through the Bunsen flame. A fairly thick vaseline is then painted round the margin of the slide. A cover-glass is similarly sterilized by passing six times through the Bunsen, care being taken not to heat sufficiently to melt it. A droplet of some sterile medium—water, broth, wort, sugar solution, &c.—is placed in the centre of the cover-glass with a sterile platinum needle. This droplet is then inoculated with the organism which is to be observed, care being taken not to add too many organisms—a few isolated organisms and all groups in each field is what should be aimed at.



often saves time first to centre the drop with the low power before examining with the immersion lens; an ink or pencil dot over the drop aids focussing. The light must be diminished by closing the diaphragm, lowering the condenser, &c. (p. 124), and artificial light is generally preferable to daylight. The central parts of the drop only should be examined, not the margin.

Instead of hollow slides, various devices may be employed to form the cell. Metal, glass, or vulcanite rings, or rings cut out of thin sheet lead, cardboard, or two or three thicknesses of paper or filter-paper may be cemented on to slides with vaseline, Hollis's glue, gold size, or Canada balsam.

The only certain method for ascertaining whether an organism is motile or not, often an important clue to its identification, is by the use of hanging-drops. Actively motile organisms may frequently be met with in a resting stage, although still alive, and various factors may bring about this condition, such as old age, exhaustion of nutriment, excessive heat or cold, electric shocks, and the like. The absence of movement of an organism in a specimen prepared from an ordinary culture, particularly if more than a day old, does not necessarily prove that it is non-motile. A hanging-drop should be prepared with a nutrient medium (the best is perhaps glucose broth) and placed under conditions of temperature &c. favourable to the growth of the organism, and examined after an interval of an hour or so, or better still at intervals of half an hour for three or four hours. In this time the old cells will revivify, and new ones will have been produced, and if the organism be a motile one, more or less active movement of some of the cells is almost sure to be observed. It is necessary to beware of two fallacies in connexion with motility—not to mistake for it the so-called Brownian movement, which is a vibratory one backwards and forwards about one point, and common to all fine particles suspended in a fluid; and

not to be misled by a flotation of the cells due to currents set up in the fluid from some cause or other—all the particles then tending to move *in the same direction*.

Another purpose for which the hanging-drop cultivation may be employed is that of obtaining a permanent record of the various phases through which an organism may pass during its development. If a number of these cultivations be made, say twenty, in an exactly similar manner, and afterwards kept under identical conditions, and if at the end of every half-hour one of the preparations be taken, its cover-glass carefully removed, and the droplet dried and stained, a permanent record of the life-history of the organism is obtained extending over ten hours.

Various more elaborate forms of cells for hanging-drop preparations can be obtained, some being provided with inlet and exit tubes for the passage of various gases. For anaerobic preparations cells are made having a groove at the bottom into which a mixture of pyrogallie acid and potash is introduced.

The observation of hanging-drop cultivations at blood-heat can be carried out on some form of warm stage.

Interlamellar Films.—Another method of investigating the life-history of organisms, especially moulds and protozoa, is by means of interlamellar films. A glass slide $1\frac{1}{2}$ by 3 inches is sterilized in the Bunsen flame, and while hot three small drops of sealing-wax are placed on it, so arranged that they form the apices of an equilateral triangle, the side of which measures about one inch, and a drop of sterile nutrient medium is deposited between them. A cover-glass of about $1\frac{1}{4}$ inch in diameter is then sterilized in the Bunsen flame, a droplet of a suitable nutrient medium is placed upon it and inoculated with the organism to be observed, and the prepared cover-glass is

¹ Delépine, *Lancet*, 1891, 1. June 13.

picked up with sterilized forceps, inverted, and lowered on to the slide. The nutrient medium is thus contained between the slide and the cover-glass, and by using a hot wire, and so softening the sealing-wax, it can be spread out to form as thin a layer as desired. The preparation is kept in a moist chamber to prevent evaporation and can be studied when required.

THE MICROSCOPE.

A bacteriological microscope should be of the monocular form, and have a rack-and-pinion coarse adjustment and an efficient fine adjustment. The stage should be large and roomy and quite plain, with two or more holes at its margin to receive spring clips for fixing the slide. For

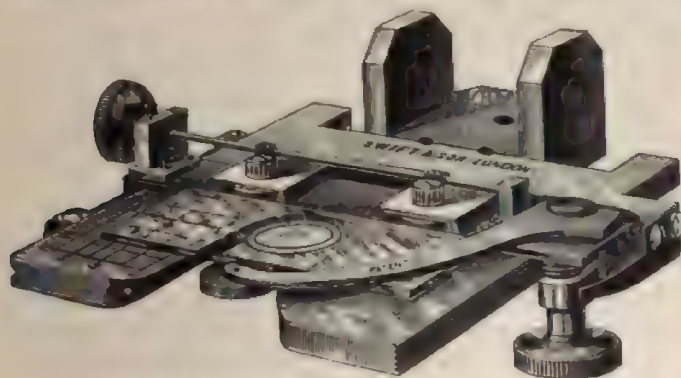


FIG. 22.—SWIFT'S DETACHABLE MECHANICAL STAGE.

the ordinary examination of specimens a mechanical stage is not needed; it hampers that freedom of manipulation which is so useful in rapidly looking through a specimen, and by practice the fingers become educated to perform the finest movements. For some purposes a mechanical stage is very useful—for systematically examining the whole of a specimen, e.g. a blood-film, it is essential. A detachable

MANUAL OF BACTERIOLOGY

to be preferred (fig. 22), so that, if required, the stage may be disencumbered for the examination of the slides used for plate cultivations &c. The stage is best made of vulcanite, which is preferable to either glass

A stage condenser is essential for all work in which objectives are employed, and also enhances the value of objectives. It consists of a system of lenses below the stage, by means of which the light is concentrated on the specimen. It should have a rack and pinion, or screw adjustment for focussing, and be provided with some form of diaphragm for modifying the light, preferably an 'Iris.' For the best results the condenser must be centred—adjusted so that its optical axis corresponds with the optical axis of the objective; and for this purpose it is provided with two lateral screws working at right angles to each other, by means of which its position relative to the optical axis can be altered. In order to

small diaphragm, or by racking down the condenser, or both; while for stained or opaque objects the full aperture of the diaphragm, or thereabouts, may generally be employed. It must be remembered, however, that the resolving power of a lens (see below) is diminished by closing the diaphragm and by throwing it out of focus; the illumination then becomes 'non-critical.' For fine work, if the illumination is too intense, this should be diminished by diminishing the source of light or by interposing a coloured screen. For this purpose Gifford's screen is an admirable one, consisting of a cell containing a solution of malachite green in which is inserted a piece of green signal glass. Coloured glass may also be interposed. The microscopist should accustom himself to examine specimens both by daylight and by artificial light; hanging-drop specimens are usually best seen with the latter. For artificial light, probably nothing surpasses a paraffin lamp with flat wick, the *edge* of the flame being always used, while to obtain the best results the mirror should be removed, and the flame used direct by elevating and tilting the microscope somewhat. For the finest work, daylight illumination is inadmissible. An admirable form of electric lamp is the 'Barnard,' made by Messrs. Swift & Son, the source of illumination being a Nernst lamp. For ordinary routine work, an incandescent electric lamp, with frosted bulb, or a Nernst lamp screened with ground glass, may be used, or an argand or incandescent gas burner. Various devices have been introduced for the employment of monochromatic illumination, e.g. the mercury electric lamp by Barnard.

Two eyepieces are sufficient, and the lower-power ones are to be preferred, such as the B and C of the English, or the 2 and 3 of the Continental makers. Although increased magnification can be obtained by the use of a high-power eyepiece, it is at the expense of definition, the image losing its sharpness, because the eyepiece magnifies the

image formed by the objective, and any imperfections in the latter are made more apparent, so that the use of very high eyepieces is not to be recommended, except with the finest lenses; moreover, as will be pointed out later, it is useless to increase the amplification beyond a certain point.

With regard to the length of the tube of the microscope, this differs in the English and Continental systems. The standard English tube-length is 8.75 inches, the Continental 6.3 inches. The latter is much more convenient for working purposes, but the finest objectives are now usually adjusted for the English length. The tube of the microscope is generally provided with an inner, or draw-tube, by means of which its length can be nearly doubled; this gives increased amplification, but at the expense of definition, at least with the higher powers which are corrected or adjusted for a definite tube-length.

The lenses or objectives must next be considered.

For powers higher than the $\frac{1}{2}$ -inch, or thereabouts, it is advisable, for many reasons, to employ the immersion system of objectives. With these lenses a drop either of water, in the water-immersion system, or of cedar oil, in the oil-immersion one, is placed on the cover-glass, and the objective is racked down so that its front lens touches and is immersed in either the water or oil, as the case may be. It is a good plan then to raise the objective very slightly by means of the coarse adjustment, still, however, keeping it in contact with the drop of water or oil. The observer then, looking down the microscope, very cautiously and gradually racks down again with the coarse adjustment until the object comes into view, and finishes the focussing with the fine adjustment. The fine adjustment should only be used after the object has been brought into view by means of the coarse adjustment. After the examination has been concluded for the day, the lens should be carefully wiped with a soft rag, or preferably with a piece of soft Japanese paper,

to remove the water or oil. If the oil should happen to dry on the lens, it may be removed by wiping with a soft rag or Japanese paper moistened with xylol, quickly drying with another rag or paper.

The immersion system of objectives has many advantages: the loss of light is less, the distance between the cover-glass and the front of the objective—the working distance, as it is termed—is greater, and more can be seen with an immersion lens than

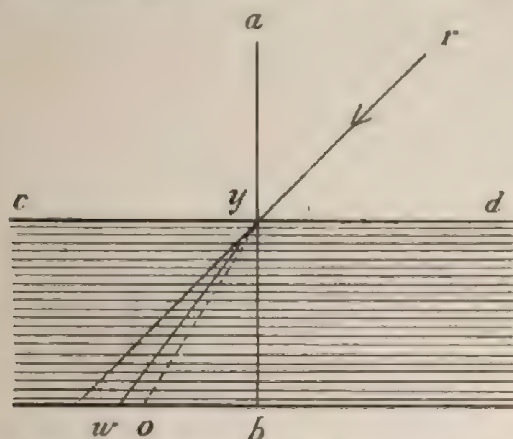


FIG. 23.—DIAGRAM TO ILLUSTRATE THE REFRACTION OF LIGHT.

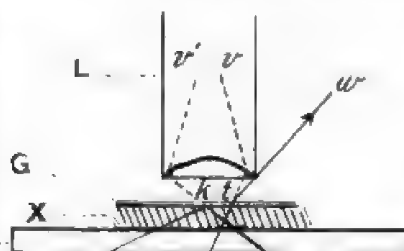
with a dry lens of equal magnifying power. This can be best illustrated by means of two simple diagrams.

In fig. 28 let cd represent the surface of a fluid, either water or oil, and let ab be drawn perpendicular to this surface, and cutting it at y . Let ry represent a ray of light proceeding from a rarer medium, such as air, into a denser one, water or oil. As is well known, this ray when it enters either the water or the oil does not continue in the same direction, but is 'refracted' or bent nearer the perpendicular ab , the bending being more marked with oil than with water. Thus we may suppose that the direction of the ray in water would be represented by the line yw , and in oil by the dotted line yo . Conversely, a ray of light proceeding

MANUAL OF BACTERIOLOGY

When a ray of light passes from a denser medium into a rarer is bent away from the perpendicular, and the rays $w y$ in water, and $v y$ in oil, would, on passing into air, proceed in the direction $y r$.

Fig. 24 (which, for convenience, is drawn somewhat out of proportion) let s represent an ordinary glass micro-slide, x a layer of Canada balsam in which the object is mounted, and covered with a cover-glass c , while L is the objective with its front lens. The object be illuminated by the ray of light $Y y$; this on passing from the glass of the slide and the Canada balsam will be refracted, or bent nearer the perpendicular and will proceed in the



former one, represented by the line tw , and misses the lens altogether—the lens is unable to take it up. If, however, we suppose that our objective is an oil-immersion one, and that a drop of cedar oil takes the place of the layer of air between the cover-glass and the front lens in the foregoing example, then the glass slide, Canada balsam, cover-glass, cedar oil, and the front lens of the objective, form practically one medium; they all have the same refractive index and produce the same amount of refraction or bending in a ray of light. Therefore the direction of the ray forms a straight line in all these, and the ray passes into the objective as is represented by the broken line $t-v$. More important still, however, is that which happens to rays which fall on the slide at a very oblique angle. In the same figure (fig. 24) let ef represent such a ray; on entering the slide it will be refracted, and its passage through the slide, balsam, and cover-glass may be represented by fk . As before, let us suppose that in the first place our objective is a dry one, and that we have a layer of air between the cover-glass and its front lens. In this case, if the angle which fk makes with the perpendicular is greater than about 39° or 40° , the ray, instead of emerging from the cover-glass into the layer of air, is totally reflected by the cover-glass and pursues a course roughly represented by $k-p$, so that it never gets near the objective. If, however, we employ an oil-immersion objective, with oil instead of air between the cover-glass and its front lens, then, as before, the slide, balsam, cover glass, oil, and front lens of the objective form practically one homogeneous whole, and the ray efk , instead of being totally reflected, continues its course in a straight line, and is taken up by the objective, as is represented by the dotted line $k-v'$. Hence we see that the same rays which are unable to enter a dry objective are taken up by an oil-immersion one, and that an oil-immersion lens can take up rays which fall on the slide at a very oblique angle.

In order that these oblique rays may be present, ready to be taken up by the oil-immersion objective, it is necessary to employ a sub-stage condenser. It is only by means of a sub-stage condenser that a 'wide-angled cone of rays,' as it is termed, is obtained. Hence to make full use of an oil-immersion objective to 'get the most out of it'—it is absolutely essential to employ

MANUAL OF BACTERIOLOGY

the condenser, and for the finest work a special 'oil-immersion condenser' is employed. It will also be obvious that a water-immersion objective admits more rays than a dry one does not admit so many as an oil-immersion. It must be noted, however, that Canada balsam, or some medium of the same or a higher refractive index, must be used for immersion to obtain the full advantage of the oil-immersion lens. The most serviceable oil-immersion lens for general use is of 1 inch (2 mm.) The oil-immersion can of course be used for mounting objects in water &c., cedar oil being still between the cover-glass and the lens. It is to be noted that a dry objective cannot be used as an immersion one, nor an oil-immersion objective dry, the construction being different in the two cases.

Aberrations in the objective are formed by cementing together different kinds of glass in order to correct for spherical and for 'chromatic' aberration. The rays passing through the margin and the centre of a simple lens are focused at the same point, and a distorted image is the

power,' or the amount of detail which can be seen. Thus, if a number of fine equi-distant parallel lines be ruled on a glass plate, it is impossible to see with a dry lens more than about 96,000 lines to the inch as isolated lines. If more are ruled they will not appear, and practically nothing is visible. With a water-immersion objective it is possible to see about 128,000 lines to the inch, and with an oil-immersion as many as 146,000 lines to the inch, as separate lines—a clear gain in resolving power in the latter case of about one-half over a dry lens. As it is necessary, in order to see such fine structures as lines ruled 50,000 or more to the inch must be, to have considerable amplification in addition to resolving power, not much is gained, in ordinary work at any rate, by adopting the immersion system for the lower power objectives, such as the $\frac{1}{8}$ -inch.

By the physical theory of microscopical visibility, it can be shown that objects having a diameter of less than about 0.16μ cannot be seen with the best optical appliances. If, then, a micro-organism is less in size than this it could not be seen microscopically, and this fact may explain why it is that in certain undoubted infective diseases no micro-organism has yet been isolated. Of the existence of such 'ultra-microscopic' organisms we have proof. The finest porcelain filters, such as the Chamberland B, do not allow visible particles to pass through, yet in several instances, if the infective material be filtered through such a filter, the filtrate is still infective. This is the case with the blood serum in yellow fever, Cape horse sickness,¹ hog cholera and swine fever, in bird and cattle plagues, and with the juice of bird molluscum. The organism of cattle pleuropneumonia is just on the limit of visibility. The rabic and vaccine viruses also seem capable of passing through a Berkefeld V. These experiments do not necessarily prove that the organism *in all stages* is invisible.² Siedentopf and Zsigmondy have

¹ *Journ. Comp. Path. and Therap.* xiii., 1900, p. 1.

² See Roux, *Bull. de l'Inst. Past.* i., 1903, pp. 1 and 49; Remlinger, *ib.* iv., 1906, pp. 337 and 385.

devised a method whereby ultra-microscopical particles may be rendered visible, but inasmuch as they appear merely as luminous points, it is questionable whether the method will be of great service in bacteriology.

There is no real necessity in bacteriological work for the immersion objective to be provided with a 'correction collar.' The 'correction collar' is an additional screw in the objective by means of which the distance between some of its constituent lenses can be altered to 'correct' for varying thicknesses of cover-glass &c., and though necessary with the higher power dry lenses, it is theoretically unnecessary with the immersion system. Nevertheless, as slight variations do occur in the various media, glass, oil, &c., and they may not form a truly homogeneous whole, for the finest work the correction collar is still desirable. So much for the high-power objectives. As regards the lower powers, which, of course, are dry, a $\frac{3}{8}$ -inch and a $\frac{1}{4}$ -inch are ones to have. The $\frac{3}{8}$ -inch is a more serviceable lens than the 1-inch, which is often recommended. A very useful accessory is a 'double' or 'triple nosepiece.' This consists of a light metal framework, which is attached to the lower end of the tube of the microscope, on to which two or three objectives can be screwed. The framework can be rotated, thus bringing each objective in succession into the optical axis of the instrument, and the necessity for unscrewing and screwing on each time an objective is changed is done away with. A microscope such as described, with sub-stage condenser, two eyepieces, a $\frac{3}{8}$ -inch and a $\frac{1}{4}$ -inch dry and a $\frac{1}{2}$ -inch oil-immersion objectives, triple nosepiece, &c., complete in case, can be obtained for about 15*l.*, and it is well to add an extra pound or two for superior finish. Both English and Continental firms supply microscopes arranged as indicated, and in this department the English makers hold their own.

The measurement of micro-organisms is carried out by

means of a stage or of an eyepiece micrometer. The former consists of a scale of tenths and hundredths of a millimetre or hundredths and thousandths of an inch ruled in fine lines on a glass plate, by means of which the measurements can be made by focussing the scale under the microscope. A useful adjunct is a disc of glass ruled with equidistant fine lines, which can be placed in the eyepiece, the value of these divisions being ascertained by means of the stage micrometer. The stage micrometer is then removed and the object to be measured put in its place, and its dimensions determined by means of the eyepiece scale. With the eyepiece micrometer, the value of the divisions is first ascertained by means of the stage micrometer, which is then replaced by the object. If the objective or the eyepiece be changed, the value of the divisions of the eyepiece scale in both cases will be altered, and must again be determined by means of the stage micrometer. The unit for microscopical measurement is the micron (sometimes erroneously termed a micro-millimetre), which measures one-thousandth of a millimetre, or $\frac{1}{25000}$ of an inch, nearly, and is designated by the sign μ .

If a micrometer is not available, rough measurements may be carried out by comparison with a red blood-corpuscle. The majority of the red corpuscles of normal human blood measure 7.5μ in diameter.

MANUAL OF BACTERIOLOGY

CHAPTER V.

—VEGETABLE AND ANIMAL PARASITES— THE INFECTIVE
—ANTIBODIES—ANTI-SERA AND ANTITOXINS—
—IMMUNITY.

INFECTION.

The term INFECTION is meant the invasion of the tissues by living micro-organisms which grow and multiply at the expense of the host. A disease produced by the growth and multiplication of micro-organisms is called an *infective disease* and is transmissible in most

and these terms have now to a large extent been given up. Excluding individual susceptibility, the relative infectivity of a disease probably depends on three factors: (1) the contagion is freely given off aërially and is not destroyed thereby, (2) the contagion gains access by the respiratory tract, and (3) the relative virulence of the contagion; in some instances the smallest amount of the contagion is sufficient to infect. If the contagion can gain access only through a wound or the digestive tract, the chances of infection may be largely reduced. In certain instances infection is conveyed by an intermediary, e.g. the mosquito in malaria, and in such cases infectivity will obviously depend on the presence and abundance of the intermediary. Infection is manifestly a part of the whole subject of parasitism, which includes the animal and vegetable parasites which develop in the animal body. If, however, the subject of parasitism is considered more closely, it will be seen that there is a vast difference between, say, a condition caused by the echinococcus or by the round worm, in which the effects are largely mechanical and in which relatively little poison is produced by the parasite, and the disease diphtheria caused by the diphtheria bacillus, in which the diphtheria bacilli have little or no action mechanically, but elaborate virulent chemical poisons which cause a general *intoxication*. Some parasites also may produce a *general* infection, e.g. anthrax, others only a *local* infection, e.g. ringworm.

Parasites may therefore be divided into infective and non-infective, though there is a series of connecting links between these, and the two groups cannot be sharply separated. The *infective parasites* are: (1) vegetable micro-organisms, chiefly bacteria, a few yeasts and some moulds; (2) many protozoa; and (3) a few metazoa, generally worms. The *non-infective parasites* are the animal parasites generally, particularly many worms.

The production of the phenomena of disease by pathogenic organisms has been ascribed to (1) the using up of the oxygen which should go to the tissues; (2) the using up of the proteins of the body and of the food; (3) the effects of plugging of the vessels by the microbes; and (4) the effects of substances or 'toxins,' having a poisonous action, formed by the microbes. Of these, the first three are quite subsidiary, embolism and thrombosis being perhaps the most important, and the toxins are the chief factors which induce the pathogenic effects. These toxins are substances of a very complex composition, probably allied to the proteins; in some instances they seem to be of the nature of enzymes or ferments, and they are direct products of the bacterial cells. The toxins of most pathogenic organisms, e.g. typhoid, cholera, plague, &c., are more or less integral parts of the bacterial cells; they are 'endotoxins,' and are not excreted to any extent into the surrounding medium. A few organisms, notably *Bacillus diphtheriae* and *Bacillus tetani*, produce extra-cellular toxins which are found in the culture liquid. The toxins are classified by Sidney Martin¹ as follows:

(1) Poisons produced by the digestive or the destructive action of bacteria on proteins in the culture medium. Examples of these are the poisons of the *Bacillus anthracis* and of the pus-producing staphylococci.

(2) Poisons which are the result of the digestive or destructive action of bacteria on proteins but formed as an excretion (the toxin) of the bacterium. The *Bacillus diphtheriae* is the best example of this. A similar combination of poisons is found in snake-venom.

(3) Poisons which are excretions only, such as those produced by the tetanus bacillus.

(4) Poisons which are typically intra-cellular, but which may also be excretory. The poisons produced by the

¹ *Manual of General Pathology*, p. 76.

typhoid bacillus, the *Bacillus coli*, the *Bacillus enteritidis* of Gaertner, and the cholera vibrio belong to this group.

THE INFECTIVE PROCESS.

With regard to the pathogenic organisms, or disease germs, Koch has laid down the following conditions, which have been termed 'Koch's postulates,' which must be complied with before the relation of an organism to a disease process can be said completely to be demonstrated:

(1) The organism in question must be present in the tissues, fluids, or organs of the animal affected with, or dead from, the disease.

(2) The organism must be isolated and cultivated outside the body on suitable media for successive generations.

(3) The isolated and cultivated organism, on inoculation into a suitable animal, should reproduce the disease.

(4) In the inoculated animal the same organism must be found.

To these may be added:

(5) Chemical products with a similar physiological action should be obtainable from the artificial cultures of the micro-organism, and from the tissues of man or animals dead of the disease.

(6) A specific serum reaction, agglutinative or bacteriolytic, or both, is generally obtainable, under certain conditions, if the blood of the infected person or animal be allowed to act on the specific organism producing the infection.

It is true that one or more of these conditions may not be fulfilled in all cases, but on general evidence the disease is classed as infective. This is the case in leprosy, for example: the *Bacillus lepræ* cannot be inoculated into animals (with the exception, perhaps, of apes), and it is doubtful if it can be cultivated, and in rabies the organism is quite unknown.

MANUAL OF BACTERIOLOGY

nodes of infection, or entrance of the infective to the body, are varied. The infective agent may—(1) the gastro-intestinal tract, e.g. typhoid, and glanders; (2) the respiratory tract, e.g. pneumonia and influenza, and occasionally typhoid, plague, &c.; inoculation, not necessarily only of the skin, but of the mucous membranes, e.g. the septic diseases, tetanus, &c. The extreme infectivity of some—e.g. variola, scarlatina, influenza, &c.—may be the fact that infection takes place by the respiratory tract. In certain instances the infection is conveyed in a special way, e.g. by mosquitoes in malaria and in fever. Nor is infection necessarily confined to one entrance; in plague, for example, infection by the commonest, but it is not infrequent by the respiratory tract. The infecting agent may remain localized, giving rise to a *local infection*, or be widespread through the body, a *septicæmia*.¹

ANTI-BODIES.

Another remarkable property, and one of considerable importance in immunity, conferred by the injection into an animal of complex substances, such as bacterial toxins, bacteria, blood-corpuscles, cells and cellular proteins, ferments, &c., is the development of *anti-bodies*. Thus an animal injected with sub-lethal doses of a bacterial toxin, such as diphtheria, acquires a tolerance towards the toxin, becomes immunised, and a substance is developed in the blood that antagonises the toxin which was injected; this substance is known as *antitoxin*. If bacteria be injected, the fresh blood *in vitro* has a solvent action on the bacteria (bacteriolysis); if blood-corpuscles be injected, the blood has a solvent action on the blood-corpuscles (hemolysis); if cells be injected, the blood has a solvent action on the cells (cytolysis), and so on. If ferments be injected, anti-ferments are formed and will prevent the specific action of the ferment. With doubtful exceptions, it is only complex bodies of protein nature, or allied to the proteins, which give rise to the production of anti-bodies on inoculation. Any substance which gives rise to an anti-body may be termed an 'antigen.' These anti-bodies &c. may first be considered, after which immunity will be discussed.

ANTITOXINS.—The anti-bodies produced by the inoculation of an animal with bacterial toxins or toxic proteins (e.g. ricin, abrin, and snake-venom) are known as antitoxins, and are of considerable practical importance. An animal injected with increasing amounts of the toxin acquires a high degree of immunity, and its blood serum injected into a second animal confers on the latter a similar immunity against the toxin with which the first animal was injected, but not against other toxins; the serum is specific. The anti-serum formed by the injection of toxin is antitoxic and not anti-microbic, and the diphtheria bacillus will grow and

multiply in diphtheria antitoxin. Since, however, such an organism as the diphtheria or the tetanus bacillus produces its pathogenic effects through the toxin which it forms, the antitoxin will counteract the effects of the *micro-organism* as well as of its toxin. The neutralization of the micro-organism, however, may not be quite complete, a certain amount of local reaction or necrosis ensuing.

Antitoxins are prepared by injecting animals—preferably horses, but goats, rabbits, &c. may also be employed—with bacterial toxins or living or dead cultures.

With those organisms which produce powerful toxins, such as diphtheria and tetanus, it is customary to grow the organism in a fluid medium so that an active and virulent toxin is obtained. The culture is then filtered through a Berkefeld or Pasteur-Chamberland filter and the toxic filtrate inoculated subcutaneously into an animal, generally a horse, commencing with small quantities.

The dose of toxin can be gradually increased, and concurrently with the increase in insusceptibility the blood serum acquires antitoxic properties. The treatment is tedious and the activity of the antitoxic serum is dependent upon the amount and activity of the toxin injected. The requisite degree of strength having been attained, the horse is bled with aseptic precautions, the blood allowed to coagulate, and the serum drawn off and bottled for use. Besides the fluid serum, a dried product is prepared by evaporating the serum to dryness *in vacuo* at 40° C. (10 c.c. serum = 1 gram dry residue), and a concentrated form may be obtained by precipitation.

The mode of production of the antitoxin by the injection of the toxin has been the subject of various theories. By some it has been supposed that the antitoxin is modified toxin, the modification being brought about by the vital activities of the cells. But the amount of antitoxin produced does not necessarily bear any relation to the quantity

of toxin injected. Woodhead records instances in which the amount of antitoxin formed amounted to 40,000 times the equivalent amount of toxin injected, and substances which increase the secretive properties of glandular cells, such as pilocarpine, enormously increase the output, so to speak, of antitoxin.

In view of these facts Ehrlich has elaborated his 'side-chain theory,' a theory which, whether it be the real explanation or no, has received a considerable amount of experimental support, and has had far-reaching



FIG. 25. — DIAGRAM TO REPRESENT THE CELL WITH ITS VARIOUS COMBINING GROUPS OR SIDE-CHAINS. (After EHRLICH.)

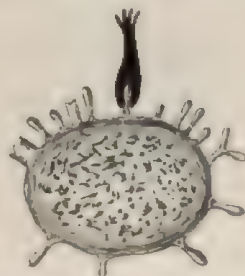
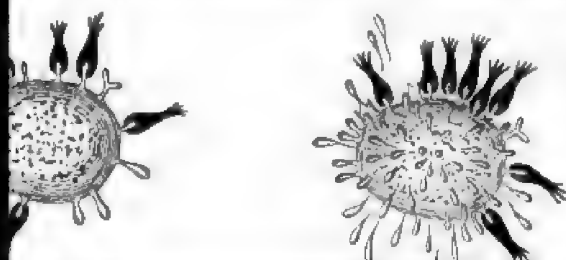


FIG. 26. FIRST STAGE IN ANTITOXIN FORMATION. (Black = Toxin molecule.) (After EHRLICH.)

effects in stimulating research. Ehrlich believes that the chemical activities which are the manifestations of the vital activities of the living cell are due to a very large nucleus or chemical molecule having a ring structure, analogous to the benzene ring, and having attached to it a number of atomic groups or 'side-chains.' A 'side chain' is an atomic group, a carbon atom of which is linked to one of the carbon atoms in a ring. These atomic groups or side-chains are unstable in nature, and enter freely into combination with other suitable groups should these be presented to them, and thus the physiological activities of the cell, assimilation, nutrition, &c., are carried out (fig. 25). Now Ehrlich

MANUAL OF BACTERIOLOGY

that antitoxin is merely an excess of certain side-chains which are normally present and subserve some of the functions of the cell and which have become free in solution. The antitoxins being specific, by this assumption the difficulty is obviated of supposing that special chemical molecules exist preformed ready to combine with any one of different toxins on the off-chance that some one may at some time or other come within the sphere of action of one of those groups. The genesis of antitoxin by the 'side-chain theory' takes place in



stimulation, as it were, the cell commences to form the particular side-chains *in excess of that needed to repair the defect created*, and ultimately these side-chains are reproduced in such numbers that they can no longer remain attached to the cell but become free in the plasma (fig. 29). *These side-chains, detached from the cell and floating free in the blood-stream, constitute the antitoxin.* This excessive production of side-chains after stimulation by repeated injections of toxin is not a phenomenon confined to antitoxin formation, but is a general physiological law enunciated by Weigert; as a result of repeated stimulation, over-production or over-compensation is the rule.

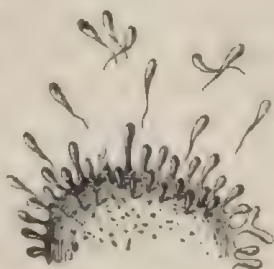


FIG. 29.—FOURTH STAGE IN ANTITOXIN FORMATION. SIDE-CHAIN, I.E. ANTITOXIN, FREE IN THE BLOOD. (After EHRLICH.)

The toxin molecule, according to Ehrlich, possesses at least two fixative atomic groups or side-chains. One of these, the 'haptophore group,' conditions the union of the toxin molecule with cell-protoplasm; the other, the 'toxophore group,' conditions its toxic action. Similarly, in order that the cell may suffer the full effect of the action of the toxin, it also must possess two receptive groups or side-chains having a maximum affinity for the haptophore and toxophore groups of the toxin; these may be termed the 'receptor' and 'toxophile' groups respectively (fig. 30). The relationship of each fixative group of the corresponding groups—viz. that of the toxin and that of the side-chains of the cell—must be of the most intimate, and analogous to the relations to each other of a male and female screw (Pasteur) or of a lock and key (E. Fischer). The existence of both haptophore and toxophore groups in the toxin molecule is suggested by the following

experiments. If tetanus toxin be injected into the blood-stream of an animal it rapidly disappears, within a few seconds of the injection, and even if the animal be at once bled, the blood withdrawn being replaced by fresh blood, tetanus ensues, but not till after the lapse of an incubation period of some hours. The tetanus toxin, therefore, immediately becomes fixed or anchored to the tissues of the central nervous system. Evidently the toxin molecule enters at once into combination with the nerve tissues by means of



FIG. 30. —DIAGRAMMATIC SCHEME TO REPRESENT THE UNION OF TOXIN (BLACK) WITH THE CELL.

In *a* the toxin is attached to the protoplasm by the union of the haptophore and receptor groups. In *b* the toxophore and toxophile groups have also united, and poisoning now ensues.

its haptophore group; this after a time brings the cells within the sphere of influence of the toxophore group, and after a certain incubation period toxic symptoms ensue. The affinity of the tetanus toxin for the nerve tissues may be shown in another way. If tetanus toxin be emulsified with fresh guinea-pig brain, the emulsion will be found to be innocuous on injection owing to a combination between the two having taken place. The cerebral cortex of a highly susceptible animal (e.g. mouse) has a marked neutralizing power, of a less susceptible animal (e.g. rabbit, fowl) a feebler, and of an insusceptible animal (e.g. frog,

tortoise) no neutralizing power.¹ Moreover, both diphtheria and tetanus toxins may be converted into non-toxic modifications which to some extent retain their power of immunizing on inoculation and of combining with antitoxin: that is to say, according to Ehrlich, the toxophore groups have been destroyed while the haptophore groups remain unaffected. It is the presence of the haptophore group which conditions the union of toxin with antitoxin. Thus, if toxin be injected into blood containing antitoxin, the haptophore groups of the toxin unite with the free receptor groups, i.e. with the antitoxin (fig. 31), and therefore the toxophore groups cannot exert their influence because the toxin is now unable to unite with the protoplasm, its haptophore or binding groups being already occupied.

In a poisonous toxin, such as diphtheria or tetanus toxin, the toxophore group is more readily destroyed than the haptophore group, and by heating a toxin for some time to 60°-70° C. its toxicity is destroyed, but it still retains an affinity for antitoxin. If some antitoxin be mixed with such heated toxin it will be found that the capacity of the former for neutralizing toxin is much diminished—in other words, although the toxophore groups of the toxin have been destroyed, the binding or haptophore groups still remain. Toxin which has been kept for some time decreases in toxicity, but retains the power of



FIG. 31. — NEUTRALIZATION OF TOXIN BY ANTITOXIN IN THE BLOOD. (After Ehrlich.)

¹ The combination of brain matter with tetanus toxin seems to be specific and of the same order as that between antitoxin and toxin. See Noon, *Journ. of Hyg.* vii., 1907, p. 101, and Besredka and Berdet, *Ann. de l'Inst. Past.* xvii., 1903.

MANUAL OF BACTERIOLOGY

ing with antitoxin, again showing that haptophore
ing groups are present (such derivatives of toxin
ing haptophore groups have been termed 'toxoids').
mann and Bruck have shown by some ingenious
ents the existence of the second stage in antitoxin
on, viz. the increased production of receptors by
s. Using tetanus toxin which had been kept
e time and had lost its toxicity, but which still
ed with antitoxin—that is, toxoids with haptophore
were still present—they found that on injecting it
imals *no* antitoxin was formed as a result of the in-

They then performed some experiments based on
owing line of reasoning: If the old non-poisonous
toxin containing these toxoids be first injected into
nal, and after a short interval some fresh, actively
as tetanus toxin, more of the active toxin ought to
red to kill this animal than a normal one, because,
o the previous toxoid injection, part of the cell

active tetanus toxin. It was found that much more toxin was required to kill this animal than a normal guinea-pig of equal size. If, on the contrary, an interval of one to three days were allowed to elapse, it was then found that a dose of tetanus toxin which would not even tetanize a normal guinea-pig was sufficient to kill this one.

The fact that no antitoxin is formed—i.e. no receptors are thrust off—on the injection of the non-poisonous toxin or toxoid Wassermann ascribes to the lack of stimulus which he suggests resides in the toxophore groups.

The slow combination of the haptophore and receptor groups has been proved by Wassermann in another way. The researches of Meyer and Ransom have shown that tetanus toxin is absorbed by the nerve trunks, not by the blood and lymph channels, while tetanus antitoxin is absorbed by the latter - the blood and lymph channels. In adrenalin we possess a substance which is able strongly to contract the capillaries, and thus to block blood absorption in a particular area. The following experiment was devised: Tetanus toxin and antitoxin were mixed in such proportions that the mixture was entirely innocuous to animals, i.e. it was just neutral. If this mixture were injected into the hind paw of a guinea-pig, no tetanus developed. When, however, some adrenalin was injected into the hind paw of a similar-sized guinea-pig, and a few minutes allowed to elapse so that the capillaries could contract, and then the mixture of toxin and antitoxin injected, typical tetanus was produced. What happened was this—the channel of absorption for the tetanus *antitoxin* had been blocked, while that for the *toxin*, the nerve path, was open. The toxin-antitoxin combination had, therefore, become dissociated, and the toxin travelled along the nerves to the central nervous system, where it produced tetanus.

The experiment, however, succeeds only within a certain

period, not exceeding an hour after mixture of the toxin and antitoxin, because after this the toxin-antitoxin combination becomes a stable one.

If a longer time—say three or four hours—is allowed to elapse, it will be found that, even in the adrenalin animal, no tetanus is produced, because by this time the combination, previously a loose one, is so firm that the substances can no longer be torn apart. This union can be hastened by employing more tetanus antitoxin, for with an excess of antitoxin, even after only half an hour, it is impossible by means of adrenalin to loosen the tetanus toxin. This experiment, therefore, shows that the tetanus toxin antitoxin combination is at first a loose one, and that the union becomes firmer and firmer with time. It also suggests the possibility of hastening the combination by increasing the dose of antitoxin, a point of considerable practical value in serum therapy.

The above considerations are of importance in the antitoxin treatment of disease. Antitoxin, in the strict sense, is not antimicrobial, and therefore antiseptic treatment of the throat in diphtheria, and of the wound in tetanus, should be pursued. The fact that the toxophore group of the toxin does not come into action as a rule for many hours at least (an exception is snake-venom) is a fortunate coincidence, allowing us to adopt antitoxin treatment before tissue damage has occurred. Antitoxin cannot repair tissue damage if this has been produced by the toxin, but it can and does prevent the occurrence of further damage by neutralizing any fresh amounts of toxin that may be absorbed. Hence the necessity for early treatment. Toxin already anchored to the tissues by its haptophore group may be withdrawn from them if a *multiple* of the simple neutralizing dose of antitoxin be injected, and the quantity necessary to accomplish this rises rapidly as the interval between the introduction of the toxin and of the antitoxin increases; hence the necessity for the use of antitoxin in large excess. Probably the union between tissue and toxin at first is a loose one, and a large amount of antitoxin by

mass action transfers the affinity of the toxin from the tissue to itself.

An essential condition in antitoxic treatment is the administration of a sufficient amount of anti-serum, and this does not depend on the actual volume of serum injected. The anti-serum may be regarded as a solution containing a variable amount of the antitoxic or anti-microbic constituent, and for therapeutic use its strength must be ascertained, and is for convenience described in arbitrary units, the units varying for different sera.

The dose of antitoxin is dependent upon the gravity of the disease, and not on the age of the patient, for a slight consideration will show that just as much toxin may be formed in a child as in an adult. The antitoxins are strictly specific; diphtheria antitoxin, for example, has not the slightest influence in tetanus.

To obtain an immediate reaction to antitoxin it should be administered intravenously. A subcutaneous injection may not be completely absorbed for two to three days.

In cases of mixed infection, e.g. where diphtheria bacilli are associated with streptococci or staphylococci, the diphtheria antitoxin will have no influence on the streptococcic or staphylococcic infection.

The complications and accidents of antitoxin treatment are few and usually unimportant. Abscess and other local troubles at the seat of inoculation should not occur if proper antiseptic precautions be taken. Urticaria or other rashes and joint pains are by far the most troublesome complications. These are due to the injection of a foreign serum, and not to the antitoxin, for the serum of an untreated horse produces a like effect. Repeated injections of serum, if a certain interval intervenes between the injections, may, however, be followed by disturbance or even grave symptoms of collapse, due to what has been termed 'supersensitisation,' and the course of events which follows the administration of two suitable injections of horse-serum differs from the sequence after one administration, e.g. in the early appearance of rash, &c.¹

Anti-sera may be used as prophylactics, but the immunity produced by them does not last more than three weeks.

¹ See Currie, *Journ. of Hyg.* vii., 1907, p. 61; Goodall, *ib.* p. 607 Grünbaum, *ib.* viii., 1908, p. 9.

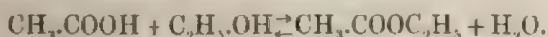
MANUAL OF BACTERIOLOGY

rious hypotheses have been advanced to explain the manner in which toxin is neutralized by antitoxin.¹ Roux and Yersin suggested that the antitoxin in some way renders the cells and tissues insusceptible to the toxin, and they performed experiments showing that while mice are more susceptible than guinea-pigs to tetanus toxin, a mixture of toxin and antitoxin which is just neutral for mice is distinctly toxic for guinea-pigs.

To explain this Ehrlich suggested that there may be present in a toxin solution, not only the toxin, but also derivatives, toxoids, and toxones (see p. 257), that have a toxic action on the guinea-pig but not on the mouse. Madsen and Dreyer showed that a mixture of diphtheria toxin and antitoxin which is innocuous to guinea-pigs by subcutaneous inoculations is lethal to rabbits on intravenous injection, and this, too, Ehrlich explained in a similar manner. Morgenroth, however, has shown that the difference in the latter case depends on the mode of injection.

all of which combine with, though they have different affinities for, the antitoxin.

Arrhenius and Madsen, however, believe that the toxin-antitoxin reaction is analogous to the action of an acid on an alcohol, and that the chemical laws of mass action apply equally to the two. The chief reaction is considered to be between two substances only, toxin and antitoxin, that it is reversible, and that when the system has reached equilibrium, a fraction of toxin and also of antitoxin remain free, this fraction of toxin producing the 'toxone effect' (see p. 258). If equivalent quantities of acetic acid and alcohol are mixed, the reaction is never complete; the acid and alcohol never entirely disappear, because the water formed reacts with the ethyl acetate, reconvertng it into acid and alcohol. Such a reaction is termed reversible, and this particular case could be thus represented:



Bordet has suggested that the fixation of toxin by antitoxin is an adsorption phenomenon, similar to the fixation of a dye by a tissue.

These hypotheses may now be examined more in detail. Ehrlich's experiments¹ on diphtheria toxin seemed to show that the neutralization of toxin by antitoxin follows the laws of simple chemical combinations, such as the neutralization of a strong base (NaOH) by a strong acid (HCl). If so, it would be expected that antitoxin would neutralize proportionate amounts of toxin; but this is not so, and Ehrlich was forced to the conclusion that toxin is a complex mixture of proto-, deutero-, and trito-toxin, and toxone, with different toxicities and different avidities for antitoxin. Moreover, when toxin is kept it decreases in toxicity, though still retaining much of its avidity for antitoxin. Ehrlich assumed,

¹ See *Trans. Jenner Inst. Prev. Med.* ii. p. 1; *Croonian Lect., Roy. Soc. Lond.* 1900; and *Refs.* pp. 150 and 257.

therefore, that the toxin becomes transformed into substances termed toxoids, which are non-toxic but retain their affinity for antitoxin. This he explained as due to destruction of the unstable toxophore groups, with the retention of the more stable haptophore groups. That the neutralization of toxin by antitoxin is due to a chemical combination between the two seems to be proved by the work of Martin and Cherry. Brodie,¹ and Martin and Cherry,² making use of a Chamberland filter, the pores of which had been rendered very fine by saturating with gelatin, found that toxin would pass through such a filter but that antitoxin would not, presumably because its molecule is much larger. By mixing diphtheria toxin and antitoxin in such proportion that the latter was in sufficient quantity to neutralize the toxin, and subjecting the mixture to filtration through a gelatin filter, the filtrate was found to be non-toxic. Now since toxin can pass through such a filter, the inference is that the toxin had combined with the antitoxin. Using snake-venom and its anti-serum or anti-venin, another method was employed. The anti-venin is destroyed by heating to 68° C. for ten minutes, while the toxic properties of the venom are unaltered by this treatment. By making mixtures of venom and anti-venin, and, after a certain time has elapsed for the interaction to take place, heating to 68° C. for ten minutes, it was found that the mixture is non-toxic, pointing to the combination of the toxin (venom) with the antitoxin (anti-venin). Calmette had performed the same experiment but with a different result, finding his mixtures still toxic after heating. Calmette, however, treated his solutions almost immediately after mixing, and Martin and Cherry point out that a certain *time* must be allowed to elapse for the interaction to take place, and noted that moderate warming hastened it, as is

¹ *Journ. of Path. and Bact.* 1897, p. 160.

² *Proc. Roy. Soc. Lond.* vol. lxiii., 1898, p. 420.

the case with all chemical interactions. For instance, they found that one mixture of venom and anti-venin allowed to interact for two minutes, five minutes, and ten minutes before heating, killed the animals in thirteen hours, fifteen hours, and twenty-three hours respectively (the control animal with the same dose of venom died in nine hours), but after fifteen minutes the same mixture rendered the animal ill but it survived, while after thirty minutes no toxic symptoms ensued.

At one time it was stated that by electrolysis of toxin small amounts of antitoxin are formed, but this is very questionable.¹ Electrolysis destroys the toxicity of toxins by the production of acids, chlorine, and hypochlorites.

Ehrlich's views have been opposed, principally on physico-chemical grounds. Thus, Danysz observed that if ricin or diphtheria toxin be brought into contact with its corresponding anti-body, the degree of neutralization depends on the manner of mixture. If the toxin be added to the antitoxin in two fractions allowing a considerable time to elapse between the additions, the mixture contains a much larger amount of free toxin than is the case when the whole (and same) amount of toxin is added at once to the antitoxin. This phenomenon, known as the 'Danysz effect,' seems inexplicable if toxin and antitoxin have relations the same as a strong base and a strong acid.

Arrhenius, Dreyer, and Madsen maintain that the phenomena observed in the toxin-antitoxin reaction are explicable on the hypothesis that the rate of reaction—avidity—of the toxin decreases as antitoxin is added, that the interaction is a slow one, and that different fractions of the toxin are progressively neutralized by the added antitoxin, but more and more slowly. On these grounds they consider that there is no reason to regard the diphtheria poison as a highly complicated body. Whereas Ehrlich considers the toxin and antitoxin to combine with great

¹ See Smirnow, *Berl. Klin. Woch.* 1894, No. 30, and 1895, Nos. 30 and 31; Kruger, *Deutsch. Med. Woch.* 1895, No. 21; Meade Bolton and Prase, *Journ. of Exper. Med.* i., 1896, p. 537.

MANUAL OF BACTERIOLOGY

analogous to the combination of a strong base with a
 acid, e.g. NaOH with HCl, these critics believe the avidity
 in for toxin to be feeble, analogous to the combination
 ammonia with boric acid, in which, as more and more acid is
 the amount of free ammonia decreases, but more and
 slowly, in correspondence with a hyperbolic curve. The
 can be calculated according to the law of 'mass action,'
 an equilibrium between

$$\frac{\text{Free NH}_3}{\text{vol.}} \cdot \frac{\text{Free H}_3\text{O}_3\text{B}}{\text{vol.}} = K \frac{(\text{NH}_4\text{H}_2\text{O}_3\text{B})^2}{\text{vol.}}$$

is the constant of dissociation. The curve of the
 tion of tetanolysin by anti-tetanolysin corresponds
 exactly to the ammonia-boric-acid curve.

as on Ehrlich's views the combination of toxin and
 would be represented by a straight line and the crude
 ns to be composed of a whole series of different toxins
 ances having an avidity for antitoxin, on this hypothesis,
 the greater part of the toxicity of toxin is removed by
 an, the latter must be added in large excess before the

that there is some doubt attaching to Arrhenius's calculations. According to Craw, the two substances most thoroughly investigated by Arrhenius and Madsen, diphtheria toxin and tetanolysin, do not admit of sufficiently exact determination, the former because of the uncertainty attaching to animal experiments, the latter because tetanolysin is a most unstable body. Working with a more stable substance, megateriolysin, he holds that the Arrhenius and Madsen equation does not apply. Again, on the addition of a small amount of antitoxin to toxin there is no decrease in toxicity (as noted by Ehrlich and attributed by him to the presence of toxoid) as there should be, and Arrhenius was thus forced to the conclusion that a second substance, epitoxonoid, is present with the toxin in diphtheria toxin. Craw denies that the toxin-antitoxin reaction is reversible, believes that antitoxin must be regarded as a colloid (and not in true solution), that the mixture therefore is heterogeneous, not homogeneous, and that the chemical law of mass action is not applicable.

On the other hand, Craw maintains that the phenomena of the toxin-antitoxin reaction, including the Danysz effect, have their counterpart in adsorption phenomena, such as occur in the staining of paper, porcelain, &c. with anilin dyes, in the absorption of substances by colloids &c. Thus, when solutions of arsenious acid are shaken up with colloidal ferric hydroxide, a portion of the arsenic is taken up by the ferric hydroxide and a portion remains in solution. Moreover, more arsenious oxide is taken up by the ferric hydroxide from dilute than from concentrated solutions; this has its counterpart in agglutination. Again, when an antitoxin is added to a toxin in just sufficient amount to produce a non-toxic solution, the amount of toxin which must then be added to constitute a fatal dose is greater than the minimal lethal dose without antitoxin. This is also found to be the case with ferric hydroxide and arsenious acid; if ferric hydroxide and arsenious acid are mixed so as to form just a non-toxic mixture, the amount of arsenious acid which must then be added to render the mixture toxic is greater than the toxic dose of arsenious acid.¹

¹ See Findlay, *Physical Chemistry and its Applications in Medical and Biological Science*, 1905.

Arrhenius¹ has recently replied to Craw's criticisms maintaining the correctness of his own interpretation, so that the final settlement of these divergent views must be left for future research.

The antitoxic constituent of antitoxin seems to be a protein body, probably allied to globulin, and, as already mentioned, the globulin content of the blood of an animal treated for antitoxin production increases in some cases. Tizzoni, by precipitating the antitoxic serum by saturation with magnesium sulphate at 30° C., obtained the antitoxin in the precipitate. Brieger and Boer² found that by adding 4 grams of potassium chloride or iodide to 10 c.c. of the antitoxic serum diluted with an equal volume of distilled water and dissolving, then adding 4 to 5 grams of finely powdered sodium chloride and keeping the mixture at 30° to 37° C. for eighteen to twenty hours, the antitoxic constituent was precipitated with a slight admixture of other protein and salts; the dried precipitate from 10 c.c. of serum weighed 0.4 gram. By precipitating the protein matter in the serum with a dilute solution of basic lead acetate to which a trace of ammonia had been added, filtering, saturating the filtrate with ammonium sulphate, filtering, re-dissolving the precipitate, dialysing and drying, a light powder was obtained which contained the antitoxic constituent; from 10 c.c. of diphtheria serum 0.06 gram was obtained.

Lastly, by diluting 10 c.c. of the antitoxic serum with five times its volume of water, and adding 20 c.c. of a 1 per cent. solution of zinc sulphate or chloride, Brieger and Boer were able to precipitate the antitoxin as a zinc compound. The precipitate is dissolved in feebly alkaline water (one drop of normal soda solution to 20 c.c. of water) and a stream of carbon dioxide gas passed through the solution. The antitoxin is found in the solution when the original precipitation has been performed with zinc chloride, but is entangled in the precipitate of zinc carbonate when zinc sulphate has been used.

ANTI-MICROBIC SERA.—If an animal be injected with increasing doses of bacteria, care being taken to keep below a lethal dose, the animal becomes gradually accustomed to the microbe and ultimately acquires a high degree

¹ *Journ. of Hygiene*, viii., 1908, p. 1. ² *Zeitschr.* . . . *Hyg.* xxi., 1896, p. 259.

of immunity, so that it is unaffected by amounts which would infallibly kill an untreated animal. Moreover, the blood serum of such a treated animal if injected into a second animal will protect the latter against a few lethal doses of the microbe, but not against a large amount. Nor is the protection afforded proportional to the amount of serum injected; for example, if 0.002 c.c. of anti-cholera serum will protect against 2 mgrm. of living cholera culture, three times as much, or 0.006 c.c. of the serum, will not protect against 6 mgrm. of cholera culture, and when a certain dose of the culture is reached no amount of serum will save the animal. The mode in which the serum acts may be studied microscopically. If cholera anti-serum and cholera culture be injected into the peritoneal cavity of a guinea-pig, and if the peritoneal contents be examined at short intervals afterwards, it will be found that the vibrios first lose their motility, then become distorted and globular, undergo solution, and finally disappear. The protection afforded by the anti-serum is therefore due to the destruction of the microbes by solution, the process being known as bacteriolysis,¹ and the bodies which bring it about being termed bacteriolysins. The reaction is known as 'Pfeiffer's phenomenon' or reaction, from its discoverer. If the serum and the microbes be mixed *in vitro* the latter are unaffected; apparently, therefore, some constituents of the living body in addition to the anti-serum are necessary for the solution of the microbes. But in 1895 Metchnikoff showed that the reaction would take place *in vitro* provided that some of the *fresh* peritoneal exudate of a normal guinea-pig were added to the mixture of anti-serum and microbes. The same year Bordet found that the addition of the peritoneal exudate was unnecessary provided the anti-serum were

¹ On Bacteriolysis see Gruber, *Harben Lectures, Journ. State Med.* 1902, February, March, and April.

MANUAL OF BACTERIOLOGY

fresh. These experiments prove that the solution of microbes is brought about by the interaction of at least two substances, one of which is present in the fresh serum of the living body, but is unstable and rapidly disappears from the serum, the other a relative stable body which remains during the process of inoculation. The former, the unstable body present in the normal animal, is usually called 'complement' (Ehrlich and Morgenroth), 'alexin' (Roux and Bordet), or 'addiment'; while the stable body produced by inoculation is known as the 'fixator' (Ehrlich), 'immune body,' 'intermediary,' 'fixer' (Gruber), 'fixateur' (Metchnikoff), or 'substance fixatrice' (Bordet). These considerations suggest an explanation of the fact that anti-microbial serum will destroy but a limited amount of living culture—viz. a certain amount of complement is present in the body, and when this has been used up no further bacteriolysis occurs. Anti-sera for the treatment of cholera, typhoid, etc., are relatively unimportant on this account.

The fluid in the peritoneal cavity is then examined microscopically half to one hour after the injection, and if the reaction be positive the organisms will be found in all stages of degeneration, being mostly converted into spherules. In this case, according to Pfeiffer, the organism is to be regarded as belonging to the same species as that by means of which the immunization of the animal, from which the blood serum was obtained, was carried out. If, on the other hand, the reaction be negative, the organisms are unaffected after being in the peritoneal cavity for an hour or so, and the organism is then considered to be different in species from that used for the immunization. Pfeiffer's reaction has been chiefly made use of to differentiate the cholera-like spirilla from true cholera vibrios and the members of the typhoid-colon group from one another.

The destruction of the bacteria by bacteriolysis is regarded by some as being brought about by osmotic changes, by others by processes analogous to digestion. During bacteriolysis the specific immunising substances and anti-bodies are used up, and for the lysis of a given quantity of bacteria a certain amount of immune serum is necessary, while after lysis has taken place the latter loses the power of dissolving bacteria. The same holds good for hæmolysis, and the facts relating to bacteriolysis and hæmolysis are almost interchangeable.

Method of Applying Pfeiffer's Reaction.—For Pfeiffer's test, the organism must be virulent, and a high-grade immune serum is necessary. If the organism is not virulent, it is spontaneously destroyed in the peritoneal cavity without the addition of immune serum. The method may be best explained in the case of



FIG. 32. — DIAGRAM TO SHOW THE UNION BETWEEN COMPLEMENT (BLACK) AND PROTOPLASM OF CELL BY MEANS OF THE IMMUNE BODY (WHITE). (After EHRICH.)

a vibrio supposed to be the cholera vibrio. The cholera-immune serum (obtained from a horse repeatedly injected with cholera culture) should possess a titre of not less than 0.0002 c.c., i.e. this amount of serum mixed with one loop (2 mgrm.) of an eighteen-hour agar cholera culture (virulent), suspended in 1 c.c. of broth, and injected into the peritoneal cavity of a small guinea-pig should cause granular degeneration and bacteriolysis of the vibrios within one hour.

Four mixtures are made—(a) one loop of an eighteen-hour agar culture of the vibrio to be tested, 0.001 c.c. cholera-immune serum, suspended in 1 c.c. of broth; (b) the same as (a), but 0.002 c.c. cholera serum; (c) the same as (a), but 0.001 *normal* serum of an animal of the same species as that furnishing the cholera serum; (d) one-quarter loop of the vibrio in 1 c.c. of broth, as a control of the virulence of the culture. These mixtures are then injected into the peritoneal cavities of four guinea-pigs of about 250 grams weight each. At intervals of thirty and sixty minutes hanging-drop preparations are made of the peritoneal fluid of each animal, the fluid being obtained by inserting a capillary pipette through a minute incision in the skin. In the guinea-pigs injected with (a) and (b), if the organism be cholera, the vibrios should show marked degenerative changes within sixty minutes, while (c) and (d) will show plenty of active vibrios. If the organism be non-virulent, two methods may be adopted for applying the Pfeiffer reaction. The first, a microscopical or *direct* method, is carried out by microscopical examination of hanging-drop specimens of the organism suspended in a drop of the immune serum to which a trace of fresh peritoneal fluid (complement) is added. If the organism is homologous with the immune serum, the bacteria are soon transformed into granules. Controls are put up at the same time with a known strain of the organism with (1) its homologous immune serum + complement, (2) non-immune serum of the same animal + complement; also of the organism being tested with non immune serum + complement. The peritoneal fluid may be obtained by injecting 3-4 c.c. of broth into the peritoneal fluid of a guinea-pig and four hours later withdrawing the fluid (now turbid with leucocytes) and centrifugalizing, or allowing it to stand on ice for twenty-four hours.

In the second, or *indirect*, method, the organism is used to prepare an immune serum by injecting an animal (e.g. a rabbit) with it, and the immune serum so prepared is tested on a known virulent strain in the peritoneal cavity of guinea-pigs in order to ascertain whether or no it brings about bacteriolysis, i.e. the Pfeiffer phenomenon.

Deflexion, Deviation, Diversion, or Blocking, of Complement.

Pfeiffer in 1895 observed that a *large* amount of immune serum might not protect an animal from the cholera vibrio, while a smaller amount with the same dose of vibrio did so. In 1901 Neisser and Wechsberg demonstrated an analogous reaction *in vitro*. They studied the effect of a bacteriolytic immune serum when varying amounts of the inactivated serum were employed. The quantity ranged from 0.0005 c.c. to 1 c.c. To each of these amounts constant volumes of normal serum and bacterial suspension were added. No bacteriolysis occurred when large and small amounts of immune serum were used, but with medium amounts bacteriolysis was complete. They explained this anomalous reaction, the absence



FIG. 33.—DIAGRAM TO REPRESENT THE CONDITION OF THE BLOOD IN WHICH THERE IS AN EXCESS OF AMBOCEPTORS. THE AMBOCEPTORS (WHITE) UNITE WITH BOTH COMPLEMENT (BLACK) AND RECEPTORS (DOTTED), SO THAT THE RECEPTORS CANNOT COMBINE WITH THE AMBOCEPTOR-COMPLEMENT GROUPS.

of bacteriolysis with *large* amounts of immune serum, as follows: When the amboceptors are in large excess, a portion combines with the complement, leaving some amboceptors free, and these free amboceptors then unite with the receptors before the activated amboceptors (amboceptors + complement) do, and thus the complement-amboceptor groups are rendered inert. The reaction is represented diagrammatically in fig. 33. Arrhenius, however, does not accept this explanation. He says: 'If we have the compounds *ea* and *ab* which may combine to form the compound *eab*, the formation of the latter depends wholly upon whether *c* has a greater affinity for *ab* than for *a*. If not, then *eab*.

formed, even if *a* is not present in excess.' (*a* = amboceptor, *e* = microbe, *b* = complement.)

AGGRESSINS.

Bail during the last two or three years has discussed the question of the relationship between bacteriolysis and immunity. He argues that there is apparently little relationship between the bactericidal properties of the body fluids and the immunity of an animal to infection through bacteriolytic processes; and points out that in rabbits immunised against anthrax there is no bacteriolytic power, the bacteria disappearing gradually as the result of phagocytic action of cells, chiefly marrow cells; that a comparison of the sera of sheep, rabbits and cattle shows great variation in their content of immune body, though the animals are almost equally susceptible to anthrax; and that in test-tube experiments a bacteriolytic serum is blocked when the conditions are approximated to those in the body by the addition of body cells to the mixture, the bactericidal properties of the serum disappear or are greatly inhibited. Kruse suggested that for infection to take place the invading bacteria must elaborate chemical substances which so act on the cells and fluids of the invaded animal that they overcome its natural resistance against infection. These substances are considered by him and Bail to be distinct from the toxins and are termed by these writers 'aggressins.'¹ The aggressins are supposed to be secreted by the living uninjured bacteria and not to be extracts, nor derived by solution, of the bacteria; they occur particularly in the fluids of pathological oedemas and exudates, and may be obtained from these by centrifugalization and sterilization at low temperatures. Bail believes that the aggressins cannot be anti-complements, anti-immune bodies, &c., but are substances heretofore unrecognized and the active substances of the infection, and he considers that in order to produce true immunity in disease anti-aggressin sera must be prepared. The following are some of the properties of these supposed aggressins: (1) sterilized aggressin with a non-

¹ See *Centr. f. Bakt. Orig.* xlii., 1906, pp. 51, 139, 241, 335, 437, and 546. Also an excellent summary by Marshall, *Philippine Journ. of Science*, ii., 1907, p. 352.

lethal dose of the corresponding organism renders the latter fatal; (2) aggressin alone is only slowly toxic, producing a prolonged illness with emaciation preceding death; (3) inoculation of aggressin with bacteriolytic serum into the peritoneal cavity suspends the action of the latter; (4) aggressin with bacteria block phagocytosis. Bail believes that the aggressins promote infection by interfering with the protective mechanism of the infected animal, particularly, if not solely, by inhibiting phagocytosis. Upon the power to produce aggressin Bail has classified bacteria into (1) true parasites which always produce aggressin, e.g. anthrax and chicken cholera; (2) half-parasites, the aggressin-producing power of which is variable, e.g. typhoid, cholera, dysentery, and plague; (3) saprophytes. The virulence of an organism does not coincide with aggressivity, and extremely virulent bacteria may be half-parasites.

Bail's hypotheses have been much criticised, particularly by Wassermann and Citron, and it is questionable whether aggressins in Bail's sense exist. The writer inclines to the view that the aggressins are identical with the true endotoxins of micro-organisms.

HÆMOLYSIS.¹—Some blood sera possess marked powers of dissolving the red blood-corpuscles of another species, and of setting free their contained hæmoglobin (e.g. goat serum dissolves rabbits' and guinea-pigs' corpuscles), and if an animal be injected with the blood-corpuscles of another species its blood serum generally acquires the property of dissolving the blood-corpuscles with which it has been injected. For example, the serum of a normal guinea-pig has no hæmolytic action upon the red corpuscles of the rabbit; but if a guinea-pig receive a few injections of defibrinated rabbit's blood, its blood serum acquires hæmolytic properties and dissolves the red

¹ See Bulloch, *Practitioner*, December 1900, p. 672. and *Trans. Path. Soc. Lond.* lii. Part 3, 1901, p. 208; Gruber, *Harben Lectures, Journ. State Med.* 1902, February, March, and April; Ehrlich, *Collected Studies on Immunity*.

corpuscles of the rabbit. This solution of the blood-corpuscles is termed 'hemolysis,' and the substances which produce hemolysis are 'hemolysins.' If the active serum be heated to 56° C. it loses its hemolysing power, but can again be rendered hemolytic or 'activated' by the addition of *fresh* normal serum; normal serum, however, rapidly losing its activating properties on keeping. It will thus be seen that there is an almost complete analogy between bacteriolysis and hemolysis, the latter being brought about by the interaction of two substances, one specific and stable produced by the injections, the 'amboceptor' or 'immune body,' and the other an unstable body present in fresh normal serum, the 'complement' or 'alexin.'

Blood-corpuscles are more tangible entities than bacteria, and are far easier to work with than the latter, and hemolysis has been the subject of a large amount of experimental work by Bordet, Ehrlich, Morgenroth, Gruber, Bulloch, and others, and the results obtained have shed considerable light upon the complex phenomena of immunity and of the actions of anti-bodies in general. Moreover, in hemolysis the globulicidal material seems to be identical with the bactericidal—that is to say, it is the complement or alexin. According to Ehrlich's view, whether it be normal or 'immune' serum (i.e. serum of a treated animal), bacteriolysis or hemolysis takes place only when the complement and amboceptor unite (fig. 32, p. 159), complement by itself having no affinity for the bacterium or erythrocyte, the combination forming the 'lysin,' which then acts. According to Gruber, however, neither bacteriolysin nor hemolysin exists as a chemical entity: the specific bacteriolytic or hemolytic action is based on the fact that the relative cells first absorb the immune body and so become accessible to the alexin. This is shown by the fact that the two substances do not combine in definite proportions—for example, the more the blood-corpuscles are laden with the immune body,

the smaller is the quantity of complement required to bring about their solution.

Many bacteria—e.g. *B. pyocyaneus*, *B. typhosus*, cholera-like vibrios—produce hæmolysins, and the hæmorrhages &c. so common in various septic diseases are probably partly due to the action of bodies of this nature elaborated by the infecting organisms.

PRACTICAL USES OF HÆMOLYSIS ETC.

1. *Hæmolysis Test*.—Some micro-organisms produce non-specific hæmolysins, others do not; this may constitute a difference between allied organisms. For instance, no true cholera vibrio seems to hæmolyse, while several cholera-like vibrios do so. The test can be applied in two ways: (a) Defibrinated rabbits' blood may be mixed with melted agar cooled to 45° C. The mixture is poured into Petri dishes, allowed to set, and when cool inoculated with the organism to be tested in such a manner that separate, well-defined colonies are obtained. After twenty-four hours' incubation at 37° C., colonies when hæmolytic are surrounded with a clear well-defined halo contrasting sharply with the dark opaque colour of the agar. (b) A young agar culture is emulsified in 4.5 c.c. of physiological salt solution, 0.1 c.c. of this suspension is mixed in a tiny test-tube with 0.9 c.c. salt solution and one drop of a suspension of well-washed rabbit or other corpuscles. After twelve to twenty-four hours, hæmolysis is apparent if it is going to take place.

2. *Fixation Test*.—A hæmolytic serum may be made a very delicate reagent for complement, and may thus serve as a test for an organism or an immune serum. Again, to take as an example a supposed cholera vibrio. If an immune serum (previously heated to 56° C. so as to destroy complement)—hæmolytic for the corpuscles of an animal, or bacteriolytic for a given micro-organism, e.g. cholera vibrio—be mixed with the red corpuscles of the same animal, or with the cholera vibrio, the corpuscles or vibrios absorb the immune body.

Bordet showed that if corpuscles or microbes containing the appropriate immune body be added to fresh non-heated

MANUAL OF BACTERIOLOGY

ment (e.g. fresh rabbits' serum), the corpuscles or microbes and the complement, so that none remains free in the liquid. If fresh guinea-pigs' serum be added to cholera vibrios which have not absorbed any cholera-immune body, the complement will not be absorbed and remains free in the liquid. The point of this is that if 'sensitized' corpuscles (i.e. corpuscles which have taken up immune body) be added to such a mixture the globules are quickly hemolysed. If, on the other hand, vibrios which have already absorbed the cholera-immune body be added to the same quantity of fresh serum, the microbes absorb the complement; and provided the amount of fresh serum is not too great, the complement is absorbed so completely that 'sensitized' corpuscles when added to the mixture are not hemolysed. If vibrios other than cholera be added to cholera immune serum, the immune body is not fixed, the complement added remains free, and the sensitized corpuscles are dissolved. The mixture of an inactivated hemolytic serum (i.e. heated to 56° C.) and homologous corpuscles (i.e. those with which the hemolytic serum was prepared) is known as a 'hemolytic system.' The method of carrying out the test is as follows: The immune

the end of that time the occurrence of hæmolysis is noted. If the organism is homologous with the immune serum, the immune body will fix the complement in tube 1 and no hæmolysis will occur; in tube 3 hæmolysis will occur because the complement remains free. Tube 2 serves as a control, and should show no hæmolysis in three hours (though if kept for eighteen to twenty-four hours hæmolysis will occur *if the organism produces hæmolysins*, apart from any action of complement). If the organism is not homologous with the immune serum, hæmolysis will occur in tube 1, because the complement does not become fixed, tubes 2 and 3 being the same as before.

The hæmolytic serum may be obtained by injecting rabbits with well-washed sheep's red corpuscles suspended in salt solution. Four doses of 5 c.c. intraperitoneally or 2 c.c. intravenously are given at intervals of a week, at the end of which period the rabbit's serum should be strongly hæmolytic. Sheep's blood may be obtained as aseptically as possible from the slaughter-house; the blood, as it runs, is caught in a sterile wide-mouthed bottle containing a coil of fine wire with which it is defibrinated by shaking. The fluid blood is then mixed with sterile physiological salt solution and centrifugalized, and the deposited corpuscles are again washed with salt solution two or three times, and finally suspended in salt solution in the proportion of 5 per cent.

3. *Antigen Test.*—See 'Syphilis.'

CYTOTOXINS.¹—Anti-sera, analogous to the hæmolysins or hæmotoxins, may be prepared which have a destructive action upon cellular elements; these are termed 'cytotoxins.' If a rabbit be injected with bull's semen, its serum ('spermotoxin') acquires the property of immobilizing the spermatozoa of the bull. The reaction is specific, but spermatolysis does not seem to occur. Similarly, by injecting ciliated epithelium into the peritoneum of a guinea-pig an anti-epithelial serum, or 'trichotoxin,' is developed. With liver, kidney, and nerve cells antibodies having a destructive action upon these cells are developed as a result of their injection. Nephrotoxin, the serum of an

¹ See Bulloch, *Practitioner*, May 1901, p. 499. (Bibliog.)

animal inoculated with an emulsion of kidney, when injected into a second untreated animal, produces albuminuria and uræmia with disintegration of the epithelium of the convoluted tubules; hepatotoxin, the serum of an animal treated with emulsions of liver, produces fatty and inflammatory changes in the liver resembling phosphorus poisoning; neurotoxin, the serum of an animal treated with emulsions of nerve tissues, produces paresis, paralysis, depression, convulsions, &c.; a leucotoxic serum, obtained by injecting leucocytes, agglutinates and dissolves the leucocytes, and so on. The formation and mode of action of these cytotoxins resemble those of the hæmolysins. It was hoped that the study and preparation of cytotoxins would open up possibilities in the way of treating such diseases as carcinoma and sarcoma, but so far this hope has not been realised.

AGGLUTINATION. If an animal be injected with cultures of typhoid or cholera, its serum soon acquires the property of agglutinating or causing aggregation into clumps of the typhoid bacilli or cholera vibrios respectively when mixed with a broth culture of these organisms. The reaction may be observed microscopically: if a hanging-drop preparation be made with a mixture of broth culture and diluted serum, the organisms lose their motility and soon become aggregated together into large masses or clumps. Macroscopically the reaction may be followed in a fine-bore tube into which the mixture of culture and serum has been introduced; after some hours the micro-organisms will have become aggregated into masses so large as to form visible flocculi, which may sink to the bottom of the tube. The substances which bring about this agglutination are known as agglutinins. Agglutinins seem to be present in small amount in normal serum; for instance, most normal human sera up to a dilution of 1 in 2 or 1 in 4 will agglutinate the typhoid bacillus and still more powerfully the glanders bacillus. They are also present in bacterial cultures; if an old broth culture of

typhoid be filtered, the filtrate powerfully agglutinates the bacilli in a fresh broth culture. The agglutination of organisms by anti-sera, though hardly specific, is usually very special; given proper precautions as to dilution, time-limit, condition of test culture, &c., an anti-serum will generally only agglutinate the homologous organism or closely allied species. That is, the anti-serum may not only agglutinate the organism with which it has been prepared, but also allied species, though usually not to the same extent; anti-typhoid serum, for example, may agglutinate not only the typhoid bacillus, but also, though to a less degree, members of the *B. enteritidis* group. As the result of infection or of inoculation with an organism, agglutinins may, however, be produced which agglutinate not only the organism of the infection but also other organisms—e.g. typhoid serum may agglutinate the *B. coli* as well as the *B. typhosus*. The agglutinins acting on the infecting organism may be termed primary or homologous, those on other organisms secondary or heterologous. In a case of double infection each organism may produce its own primary agglutinin, so that the agglutination of two species by a serum may be due to the presence either of a primary and a secondary agglutinin or of two primary agglutinins. Castellani¹ found by applying the saturation test (p. 175) that an organism would absorb both its primary and secondary agglutinins, but would not absorb two different primary agglutinins. This test, therefore, would distinguish a double infection from a single one. Thus, if with a typhoid serum agglutinating both the *B. typhosus* and the *B. coli*, the serum after saturation with typhoid culture still agglutinated the *B. coli*, it would point to an infection with the latter as well as with typhoid. The agglutination reaction is made use of practically in two ways in bacteriology and medicine. The 'Bordet-Durham' reaction consists in testing

¹ *Zeitschr. f. Hyg.* xl., 1902, p. 1.

an unknown organism with a specific anti-serum prepared by injecting an animal with a known microbe; if the organism tested becomes agglutinated, it is regarded as being of the same species as that with which the anti-serum was prepared. With certain precautions the Bordet-Durham reaction is one of the most delicate and certain for diagnosing bacterial species. The converse of this is the agglutination reaction proper (frequently termed the Widal reaction), and consists in testing an unknown serum upon a known microbe. It is especially used in the diagnosis of microbial diseases; for example, in typhoid fever the blood of the typhoid patient powerfully agglutinates the typhoid bacillus, that of Malta fever the *Micrococcus melitensis*, that of bacillary dysentery the dysentery bacillus, &c.

A remarkable phenomenon observed in connexion with agglutination, which the writer has particularly noticed in the case of Malta fever, is the occurrence of a negative phase with some particular dilution. Thus, dilutions of 1 in 10 and 1 in 20 may agglutinate strongly, a 1 in 30, however, may hardly agglutinate at all, while dilutions of 1 in 40 and upwards to 1 in 100 or more may agglutinate well.

Anti-serum, prepared by injecting erythrocytes, also agglutinates the red blood-corpuscles, and in certain diseases, e.g. pneumonia, chromocyte clumping may be a marked feature.

Various theories have been propounded to account for the phenomena of agglutination.

1. Pfeiffer and Emmerich and Löw regarded agglutination as a vital paralysis of the bacilli due to the action of a bacteriolytic enzyme. Agglutination, however, is not a vital phenomenon, and bacteriolytic enzymes seem to be destroyed by temperatures at which the agglutinins remain unaffected.

2. Gruber, Dineur and Nicolle supposed that a glutinous

substance, 'glabrificin,' was absorbed from the serum by the bacilli causing the cell membranes or the flagella to become adhesive; but this explanation will hardly account for the aggregation of non-motile organisms.

3. Paltauf and Duclaux considered that a specific precipitate is produced in the medium, which during flocculation mechanically carries the bacilli with it; but there is no demonstrable evidence that such precipitation occurs.

4. Bordet separated the mechanism of agglutination into two stages—(1) fixation of agglutinin, and (2) aggregation. The fixation of agglutinin by the organisms he considers to be analogous to the fixation of a dye by a tissue; and once the agglutinin is fixed, the organisms obey the laws of inert particles, aggregation being caused by changes in the molecular attraction between the organisms and the surrounding medium. This is the view which Craw¹ supports. Ohno,² however, expresses the opinion that the union of agglutinin and agglutinable substance is not analogous to the fixation of a dye by a tissue, but that it is a chemical reaction as maintained by Ehrlich.

Agglutinated bacteria are not injured by agglutination; they will, in fact, grow and multiply in an agglutinating serum. The amount of agglutination does not bear any constant ratio to the intensity of an infection; on the whole, if the organism is reacting satisfactorily to an infection, the agglutination reaction tends to be marked; if not, it may be feeble or absent. Thus, in severe typhoid infections with fatal issue, agglutination may be absent. Ruffer and Crendiropoulou³ regard the agglutinins as being formed in the polymorphonuclear leucocytes.

¹ *Journ. of Hygiene*, v., 1905, p. 113. See also Joos, *Zeitschr. f. Hyg.* xxxvi, p. 422, and *ib.* xl, p. 203.

² *Philippine Journ. of Science*, iii., 1908, p. 47.

³ *Brit. Med. Journ.* 1902, i, p. 821. (Lilbhog.)

THE AGGLUTINATION REACTION.

A. *For Clinical Diagnosis* ('Widal' Reaction).

This is principally made use of in typhoid and paratyphoid fevers, Malta fever, and bacillary dysentery.

Collection of Blood.—Blood is collected (p. 117), preferably in a Wright's capsule (fig. 34, d, p. 193), or in a capillary bulbous pipette (fig. 6, p. 14), or in a vaccine tube. The ends of the tube are sealed, the dry end always being sealed first, the blood allowed to coagulate (which may be hastened by placing in the blood-heat incubator), and then centrifugalized to separate the serum, care being taken that the dry sealed end of the tube, which will be perfectly sealed, is distal when spinning.

If tubes are not available, the blood may be spotted on to a piece of glass, cover-glass, or slide, glazed paper, tinfoil, &c., and allowed to dry. For use, a drop of distilled water is placed on the dry blood to dissolve it, and the solution used like serum.

The Culture.—For the *microscopic* test a young broth culture is preferable. It should be examined in hanging drop to see that clumps are absent; this specimen is kept as a control. If clumps are present, they may be removed (in the case of typhoid) by filtering the culture through filter-paper. A suspension of an agar culture may also be used, likewise dead cultures: a broth culture or suspension of an agar one being heated to 65° C. for ten minutes and preserved in sterilized glass pipettes.¹ For the *macroscopic* test, a thick suspension of an agar culture in salt solution is to be preferred, the suspension being allowed to sediment for half to one hour before use. Some strains of an organism are better than others.

Dilution of the Serum.—This may be carried out in various ways, with the haemocytometer pipette, with a pipette made out of a piece of glass tubing as for opsonin work (fig. 34, a, p. 193), or with a platinum loop. With the pipette a little serum is allowed to run up so as to occupy 1½-2 cm. of the stem, and the upper limit is marked with a grease pencil or ink. The pipette is then tilted so that a little air-space is left between the end of the pipette and the lower end of the column of serum. The end of

¹ See Delépine and Wright, *Brit. Med. Journ.* 1897.

the pipette is then immersed in a watch-glass of salt solution, holding it nearly horizontal (or using a test to suck up with, as in the opsonic method), and the salt solution is allowed to run up to the mark, when the process is repeated; so finally the pipette contains 1 volume of serum and 4 volumes of salt solution, each volume being separated from the next one by an air bubble. The contents of the pipette are then blown out into a watch-glass and thoroughly mixed, and further dilution of this dilution is prepared in the same manner. Two or three dilutions are usually made—viz. 1 in 15, 1 in 25, and 1 in 50. A platinum loop may also be employed as a measure; a loopful of the serum is deposited in a watch-glass, and by spotting round it nine or fourteen loops of salt solution a dilution of 1 in 10 or 1 in 15 is prepared, or any other dilution in a similar manner.

The Microscopic Test. Two or three hanging-drop slides are vaselined and two or three cover-glasses cleaned. On to each cover-glass one loopful of a dilution of serum is placed, and to each is added a loopful of the broth culture of the organism—e.g. typhoid—and well mixed up, and each specimen is mounted as a hanging drop. Starting with three dilutions of serum—e.g. 1 in 15, 1 in 25, and 1 in 50—the dilutions in the specimens will be 1 in 30, 1 in 50, and 1 in 100 respectively. Or if only one dilution of serum has been made—e.g. 1 in 15—if on each cover-glass one loopful of this be placed, and to the first be added one loopful, to the second two loopfuls, and to the third three loopfuls of typhoid culture, then the final dilutions in the three specimens will be 1 in 30, 1 in 45, and 1 in 60 respectively.

Care should be taken that the hanging-drop cultures are quite sealed with the vaseline, so that evaporation is prevented. The hanging drops are then examined microscopically, a $\frac{1}{4}$ -inch objective sufficing for typhoid. In the case of typhoid the following phenomena will be observed. The motility of the majority of the bacilli is instantaneously or very quickly arrested, and in a few minutes they begin to aggregate together into clumps, and by the end of the half-hour there will be very few isolated bacilli visible. In less marked cases the motility of the bacilli does not cease for some minutes, while in the least marked ones the motility of the bacilli may never be completely arrested, but they are

MANUAL OF BACTERIOLOGY

more or less sluggish as compared with the control drop made from the culture, while clumping ought to be distinct by the end of one hour (with a 1 in 30 to 1 in 50

central portions of the drop should be examined, not the sides. With blood which has been dried and dissolved, the organisms may become entangled in debris and must not be mistaken for clumps.

In cases two or three different dilutions should be made to detect a possible negative phase with some particular dilution.
Microscopic Method, or Sedimentation.—The serum, having been diluted by means of a pipette with four times its volume of distilled water, is mixed with 5 to 20 times its volume of culture containing plenty of micro-organisms in the same manner as described in the previous section. The mixture is placed in a fine, but not capillary, bore tube. This is sealed at the lower end and allowed to stand in the upright position for from twenty-four hours at 20° C. or six hours at 37° C.; when it is often distinct within an hour at 37° C. When the result is positive the organisms become agglutinated, and form

a rabbit three to five intravenous injections at intervals of seven days of killed culture of a virulent strain of the organism, e.g. typhoid or cholera. The culture is killed by heating to 60°-65° C. for half an hour, and the dose is increased from one loop to ten loops of an agar culture. Seven days after the last dose, the animal is bled, and the serum obtained. The agglutinating value of the serum must be determined, and controls should always be put up with normal serum of an animal of the same species as that from which the immune serum has been obtained. A series of dilutions of both sera is made with salt solution and a twentyfour-hour agar culture of the organism to be tested used. Both the macroscopic and microscopic methods should be employed. The dilutions may be made with a 1 c.c. pipette graduated in hundredths, with the hæmocytometer pipettes, or by the method used clinically.

2. *Saturation Test*.—Bordet first noticed that a suspension of a microbe added to the homologous agglutinating serum absorbs most, if not all, the specific agglutinin, whereas an organism not homologous with the serum absorbs little or only a portion of the agglutinin. The test may be carried out as follows :

(a) Ten loopfuls of a young agar culture of the organism to be tested are mixed with 10 c.c. of a 5 per cent. solution of a highly agglutinating serum. After standing for two or three hours at room temperature, the mixture is centrifugalized and the clear supernatant fluid decanted.

(b) The agglutinating power of the decanted liquid is then tested on the organism with which the serum was prepared. If the organism treated in (a) is homologous with the organism with which the agglutinating serum was prepared, the decanted fluid will have lost most, or a considerable proportion, of its agglutinating power for the latter.

ANTI-FERMENTS.¹—By the injection of rennin or other enzyme the blood serum of the treated animal acquires the property of neutralizing the action of the enzyme with which the inoculation has been performed. Thus if rennin and anti-rennin (the serum of an animal injected with rennin) be mixed with milk

¹ See Deann, *Trans. Path. Soc. Lond.* lii., 1901, Part 2, p. 127.

no curdling takes place. Similarly, the serum of an animal inoculated with pancreatin inhibits the action of this ferment, and if coagulated egg-albumin, pancreatin, and anti-pancreatin be mixed, the egg-albumin undergoes no digestion.

PRECIPITINS.¹—Kraus was the first to demonstrate the presence of specific precipitins in blood by adding typhoid, cholera, and plague anti-sera to filtrates of the cultures of the corresponding microbes. If to such a filtrate in a test-tube a little of the corresponding anti-serum be added by running in carefully, so that it forms a layer at the bottom, an opalescent ring makes its appearance at the line of junction of the two fluids. So also if an animal be injected with milk, its serum, when added to milk of the same kind as that with which it has been injected, causes precipitation of the casein. This reaction is specific, and it is thus possible to distinguish various milks from one another. Similarly, anti-sera which produce precipitates, each with the homologous substance, are obtained by the injection of peptone, of egg-albumin, blood serum, and other proteins. The latter reaction bids fair to have an important medico-legal application, for by means of it the blood and flesh of different species of animals can be distinguished. Thus the presence of horseflesh in sausages can be detected. The method employed is to inject a rabbit intraperitoneally with four to six injections of defibrinated blood or of blood serum (or with a solution of the particular substance, e.g. horseflesh), commencing with about 5 c.c. and increasing to 10 c.c. at intervals of a few days. After treatment the blood is sucked up into a pipette from one of the veins of the ear, allowed to coagulate, and the serum so obtained. The blood to be tested may be dried on filter-paper, pieces are then cut up, a solution made in 1·6 per cent. sodium chloride solution, and to this the specific serum is added. Tested in this way human blood anti-serum reacts - i.e. forms a precipitate—markedly with human blood, less so with ape's blood, not at all with other blood; ox blood anti-serum reacts with ox blood, less so with sheep, feebly with horse, hardly at all with dog. Mixtures of bloods may also be tested. The production

¹ See Nuttall, *Journ. of Hyg.* i. No. 3. 1901. p. 367 (Bibliog.), also *Brit. Med. Journ.* 1902, i. p. 825.

of the anti-body seems to be due to the globulin constituent of the injected serum.

It will thus be seen that the anti-bodies which result from the injection into an animal of different substances are extremely numerous and have varied properties, their most notable characteristics being their extreme specificity and the extraordinary delicacy of the interactions produced by them. It is important to note that these anti-bodies are produced only as the result of inoculation with complex compounds allied to the proteins. The tolerance established by the ingestion or inoculation of simpler compounds, such as arsenious acid and morphine, is of a different nature, and is not coincident with the development of anti-bodies. According to Ehrlich, the latter kind of tolerance may be due to the exhaustion or using up of certain receptors ('chemo-receptors') of the protoplasm (see p. 185).

IMMUNITY.¹

No fact in biology is more striking than the differences in susceptibility to disease conditions exhibited by different races and different animals. For example, the native races in many parts of the world are comparatively insusceptible to yellow and typhoid fevers and malaria, the dog and goat are rarely affected with tuberculosis, and tetanus is never met with in the fowl; and to come nearer home, while some individuals are lucky enough to escape most of the commoner infectious fevers, others seem to contract them on every possible occasion, and to suffer from all the ills that flesh is heir to. These instances show that there is often a natural insusceptibility to infective disease, or a natural immunity, as it is termed, either complete or partial and appertaining

¹ See Metchnikoff, *Immunity in Infective Diseases*, 1905. Also *Brit. Med. Journ.* 1902, i. p. 784; 1904, ii. pp. 557-582, and 1907, ii. pp. 1409-1425; *Journ. of Hygiene*, ii. 1902.

MANUAL OF BACTERIOLOGY

race, 'racial immunity'; or, varying in different individuals and at different ages, 'individual immunity,' as in the case of diphtheria and scarlatina, which become more and more rare as age advances.

Still more striking, perhaps, is the fact that an insusceptibility may be acquired either after an attack of infective disease or in certain instances by inoculation. Thus repeated attacks of smallpox and scarlatina are rare, while vaccinated smallpox and vaccinia protect against variola. These are examples of an acquired insusceptibility or acquired immunity.

With regard to the immunity of native races to certain diseases, this is probably due to natural selection and heredity; during long periods of time, the individuals being exposed to the same risks, the susceptible ones are eliminated, while the survivors transmit their insusceptibility to their descendants; but this, of course, does not

is, infection can usually be induced under certain conditions. Thus fowls, which are highly refractory to tetanus and tolerate considerable doses of tetanus toxin with impunity, can be tetanised with large doses of an active toxin: white rats, which are insusceptible to anthrax, become susceptible after fatigue, or when fed on an exclusively vegetable diet. Behring would ascribe the immunity of white rats to anthrax to the high alkalinity of their blood, and claims to have shown experimentally that a vegetable diet reduces this, and fatigue is said to act similarly.

Ehrlich's side-chain theory suggests a simple explanation of certain cases of natural immunity. In order that a bacterial toxin or endotoxin may produce intoxication it must become anchored to the cells by its haptophore group, and that this may occur the cell molecules must possess atomic groups or side-chains ('receptor groups') which have a special affinity for the haptophore groups of the toxin. Should these be wanting the toxin cannot become anchored to the cells, its toxophore groups cannot exert their influence, and natural immunity is the result.

The blood, lymph, and other fluids and tissue juices undoubtedly exert a more or less germicidal action on bacteria experimentally *in vitro*, and to some extent probably also in the body. But in this respect there is often a marked difference between the circulating blood and the blood *in vitro*.

Lewis and Cunningham (1872), Traube and Gscheidlen (1874), Fodor (1877), and Wysokowicz showed that bacteria injected into the circulation rapidly disappear, and were inclined to attribute this result to the bactericidal properties of the blood. In the main, however, this disappearance is probably due to lodgment in the capillaries, phagocytosis, and excretion by the excretory glands.

Halliburton prepared from the lymphatic glands a cell-

MANUAL OF BACTERIOLOGY

8. Hankin found that this had marked germicidal properties, and concluded that this substance was the germicidal constituent of the blood serum. The germicidal constituents of the cells and body fluids gave the name 'alexins.' Bitter, who repeated the experiments, failed, however, to confirm them.

Mann performed the first experiments with extra-blood. He found that anthrax bacilli after being in plasma became less virulent. Fodor, adding anthrax to blood and plating at intervals, found there was a progressive diminution in the number of organisms.

Ell in 1888 used the defibrinated blood of several rabbits, mice, pigeons, sheep, and found that it killed the *B. anthracis*, *B. subtilis*, *B. megaterium*, *B. pyogenes* var. *aureus*. He confirmed Fodor's results which also showed that after a while the blood loses its germicidal properties and becomes a suitable culture

siderable difference between the action of circulating and of extra-vascular blood.

Vaughan, Novy and McClintock in a series of papers ascribed powerful bactericidal properties to the nucleins, and surmised that in serum the nucleins set free by the disintegration of leucocytes and other cells are the germicidal agents. Forrest and the writer¹ found, however, that all the germicidal properties ascribed by Vaughan to the nucleins were probably due to the weak alkali in which the nucleins were dissolved, and came to the conclusion that Vaughan's results were at least not proven.

Gengou, also, by collecting the *plasma* in vaselined tubes, found it often to be almost devoid of bactericidal power, whilst the corresponding *serum* is capable of destroying large numbers of micro-organisms.

We therefore see that while the blood, lymph, and other fluids and tissue juices undoubtedly exert more or less germicidal action on bacteria experimentally *in vitro*, there is often a marked difference between the circulating blood and the blood *in vitro*, in this respect, and it must be doubted if this factor is of great importance in the production of natural immunity.

When, however, the organism is actually invaded and tissue damage and cellular disintegration have occurred, it is conceivable that germicidal substances are set free and help in getting rid of the invaders (though such substances may act rather by stimulating the leucocytes, as will be referred to later, p. 188).

Thus Kanthack and Hardy found that the coarsely granular oxyphile leucocytes in the frog are first attracted to the site of a bacterial invasion, there discharge their oxyphile granules, the bacteria then show signs of degeneration, and polymorphonuclear leucocytes and other 'phagocytic' cells now approach and ingest the degenerate

¹*Journ. Roy. Army Med. Corps.*

MANUAL OF BACTERIOLOGY

The observations, however, do not seem to be confirmed. Wooldridge also protected animals from anthrax by injections of 'tissue fibrinogen' (nucleo-

The hypothesis which ascribes immunity to the chemical action of alexins in the fluids of the body has given rise to the 'humoral theory.'

Another important theory of immunity is the doctrine of phagocytosis, so ably supported by Metchnikoff. This is the 'cellular' theory of immunity. It has as its basis the following fundamental facts: Firstly, the leucocytes in circulating blood ingest and destroy any foreign particles present therein; secondly, an injury to the tissues is immediately followed by an inflammatory reaction, in which leucocytes emigrate from the vessels by diapedesis and collect at the injured spot. Similarly, in many cases the leucocytes rapidly congregate at the seat of a bacterial infection, and approach and engulf the bacteria in much the same manner as they do other foreign particles, and so

however, a weak acid be substituted for the peptone water, the bacilli will be repelled. The process by which the bacteria are ingested by the leucocytes can be similarly watched. The leucocytes which act in this manner are termed phagocytes, and they are of two classes the macrophages, the large mononuclear leucocytes, and the smaller microphages, or polymorphonuclear leucocytes. Certain of the tissue cells and endothelial cells also possess phagocytic properties. That an acquired immunity may be due to the education of the leucocytes seems to be shown by the fact that, while in ordinary susceptible rabbits infection with anthrax is followed by a feeble phagocytosis and the animals succumb, in rabbits vaccinated against anthrax phagocytosis is very active. Moreover, in an animal refractory to anthrax, such as the frog, if the bacilli be enclosed in minute bags of paper or membrane, so as to prevent the access of the phagocytes, the bacilli grow and multiply. An immense amount of experimental proof has been obtained by Metchnikoff and his pupils of the truth of the doctrine of phagocytosis; but in spite of their ingenuity, the theory has not a universal application, and phagocytosis *alone* will not explain some cases of immunity, particularly against toxins. However, as Metchnikoff observes, under natural conditions it is against the penetration of the *micro-organisms* that the animal must be protected.

Metchnikoff has so far modified his theory as to admit that destruction of bacteria in phagocytosis is brought about by chemical bacteriolytic substances, which he terms 'cytases,' and which he regards as being derived from the leucocytes, and to be identical with the alexins. He believes that there are two kinds of cytases, one 'macrocytase,' obtainable from tissues, such as the spleen and lymph glands, rich in macrophages, which acts specially on elements of animal origin, the other 'microcytase,' derived from the microphages, and which acts principally on micro-

MANUAL OF BACTERIOLOGY

. He considers the alexic action to be of the digestive process (but this is doubtful), and as the complex nature of a cytolytic serum, which is a phagocyteceptor and complement, believes that the serum is produced from the macrophages in intracellular digestion and that a portion of it escapes into the blood. All the facts point to the leucocytes and tissues being the great defensive mechanisms against parasitic invasion, either by the production of antibodies by phagocytosis, or by a combination of the two (the 'cell-humoral' hypothesis of immunity). Artificial immunity may be induced in several ways :
1. By an attack of the disease ending in recovery.
2. By vaccinating with a modified and less virulent form of the infective agent (Pasteur's method).
3. By one or more treatments with sterilized cultures, or with attenuated toxins.
4. By the injection of the blood serum from an animal treated

could not again grow in the animal which had been attacked. This hypothesis, therefore, presupposes that in the body there is some nutrient material necessary for the growth of each species, which is difficult to believe, and is negatived by the fact that an organism will grow in the blood and tissues removed from an animal vaccinated against, and insusceptible to, the disease produced by it.

Pasteur's 'exhaustion' theory has been revived by Ehrlich¹ in a modified form, under the name of 'atrepsy,' to explain certain cases of immunity. Thus, for a chemical poison to act, Ehrlich assumes that particular receptors in the protoplasm for binding the poison are necessary; these he terms 'chemo-receptors.' Bird-pox, virulent for both fowl and pigeon, if passed through the pigeon becomes completely avirulent for the fowl. To explain this, Ehrlich suggests that the parasite in passing through the pigeon has to assimilate substances different from those assimilated during its passage through the fowl; therefore that part of the receptors which deals with the nutritive substances of the fowl's organism is not in use during the passage through the pigeon, and may become atrophied, so that on the parasite being transferred back to the fowl it would not be able to thrive owing to the loss of the receptors necessary to assimilate the fowl's nutritive substances. Ehrlich suggests that the majority of non-pathogenic micro-organisms, if introduced into the animal body, perish by this mechanism. In the case of mouse carcinoma inoculated into rats, the tumour cells proliferate for a few days, then atrophy and disappear. Ehrlich suggests that some specific substance is necessary for the proliferation of mouse carcinoma cells which is not present in the rat, and as soon as the traces of this specific substance carried over by the inoculation are used up, the cancer cells cease to proliferate and finally atrophy and disappear. These are examples of Ehrlich's 'atrepsy' and 'atropic immunity.'

Chauveau in his retention theory suggested that the bacteria during their growth in the tissues formed substances

¹ Harben Lecture, ii., *Journ. Roy. Inst. Public Health*, 1907.

which ultimately inhibited their growth, and, if the animal recovered, prevented a subsequent development of the organism.

The 'education' of the leucocytes whereby they are attracted, instead of being repelled, by a bacterial invasion, may be the result of an attack of infective disease ending in recovery, and so an acquired immunity from the particular infection may be brought about.

Ehrlich by his classical experiments with abrin and ricin, two toxic proteids obtained from the jequirity and castor-oil beans respectively, showed that acquired immunity is of two kinds, one 'active,' as he termed it, of long duration, and resulting from an attack of the disease or from vaccination with a modified virus and not transmissible to the fetus; the other, 'passive immunity,' resulting from the inoculation of an animal with the blood serum (antitoxin or anti-microbial serum) derived from another animal immunized by the injection of bacterial toxins or bacteria. 'Passive immunity' is soon lost, but while present is transmitted to the fetus.

Acquired immunity may be induced by inoculation with increasing quantities of a bacterial toxin, as in the treatment of an animal for the production of antitoxin. The animal finally acquires a high degree of immunity, which is transmitted to the fetus through the blood of the placental circulation and to the nursing through the milk.

Natural immunity in most cases is certainly not due to the presence of antitoxins; for example, the blood of the fowl, a bird which is highly insusceptible to tetanus, has not the slightest antitoxic power towards a tetanus toxin. Moreover, in the preparation of an antitoxin by injecting an animal with toxin the immunity towards the toxin gradually increases, and *pari passu* the antitoxic power of the serum increases up to a certain point, but then slowly and steadily falls, until it is almost lost, yet the animal remains as highly immune as ever.

It seems, however, that in certain cases the blood serum has some antitoxic action, although the individual has never suffered from the disease, and this may render him less susceptible to infection.

Thus Wassermann¹ found the blood of some healthy persons antitoxic as regards diphtheria, and Orlowski² observed the same thing in a few children, a previous attack of the disease having been excluded as far as possible. That such individuals have not necessarily passed through an attack of diphtheria seems proved by the observations of Meade Bolton³ and of Cobbett,⁴ both of whom have met with horses whose blood was definitely antitoxic as regards diphtheria.

The spleen may have some relation to immunity, for experiments by Tizzoni and Cattani seemed to show that rabbits could not be rendered refractory to tetanus by injection of tetanus antitoxin after its extirpation; and although Benario denies this, the manner in which the spleen is attacked in such diseases as tuberculosis, plague, &c. points to this conclusion.

According to Ehrlich, the progeny of an immune father (immunized by the injection of toxin) and a normal mother are not refractory—if anything, are more susceptible than control animals; but with immune mothers and susceptible fathers the immunity is transmitted. Some experiments by Tizzoni and Cattani point to the occasional transmission of some degree of immunity towards rabies through the father; such immunity is permanent and contrasts with the transient immunity transmitted through the maternal blood or milk.

To sum up, natural immunity is probably due to a number of factors, some or all of which may be operative

¹ *Deutsch. Med. Wochenschr.* 1894.

² *Ibid.* 1895.

³ *Journ. of Exper. Med.* i., 1896, p. 543.

⁴ *Journ. of Path. and Bact.* iii., 1895.

MANUAL OF BACTERIOLOGY

For instances, and it is impossible to state with any general law. In some cases phagocytosis is a means of defence, in others the fluids of the body exert a germicidal or inhibitory influence upon the bacteria; in others, again, the cells and tissues for the most part are unaffected by the bacterial toxins, perhaps because the cells are lacking in the particular side-chains which have a special affinity for the toxins.

As regards the immunity acquired after an attack of disease, this may be due to the 'education' of the leucocytes, so that they are attracted, whereas formerly repelled, by the products of bacterial development, or to substances which regulate the action of the leucocytes. The germicidal and inhibitory actions of the body fluids may also be due to the same cause. There is also a possibility that the side-chains of the toxins having an affinity for the toxin become inactivated, destroyed or used up, so that further fixation of the cellular toxin cannot take place.

although Leishman¹ attributed a stimulin action to thermostable substances in the serum in typhoid and Malta fevers. Subsequently Metchnikoff conceived the serum as acting not on the leucocytes but on the microbe, causing it to become positively chemotactic and no longer to repel, but to attract the phagocytes. Considerable support was given to this view by the work of Wright and Douglas, who, by a modification of Leishman's ingenious method for quantitatively estimating phagocytosis, emphasized the importance of the serum in the mechanism of phagocytosis.

*Leishman's Method for Estimating Phagocytosis.*²—A thin suspension of some micro-organism, e.g. *M. pyogenes*, is mixed with an equal volume of blood from the finger, a droplet of this mixture is placed on a clean slide, and covered with a cover-glass, and the preparation is at once placed in a moist chamber in the incubator at 37° C. for half an hour. At the end of this time it is taken out, the cover-glass slipped off, and the films on slide and cover-glass are dried, fixed, stained, and examined microscopically, and the number of microbes ingested by the polymorphonuclear leucocytes counted.

Wright and Douglas³ found that washed leucocytes without serum are non-phagocytic, but become so on the addition of normal serum. If, however, the serum be first heated to 60°–65° C. before being added to the mixture of leucocytes and microbes, phagocytosis does not take place; but if the unheated serum is mixed with the bacteria, the mixture kept at 37° C. for fifteen minutes and then heated to 60° C. for fifteen minutes, phagocytosis can still take place, thus demonstrating that the serum acts in some way on the bacteria, rendering them suitable prey for the phagocytes. This thermolabile serum feast preparer is

¹ *Trans. Path. Soc. Lond.* lvi., 1905, p. 344.

² *Brit. Med. Journ.* 1902, i. p. 73.

³ *Proc. Roy. Soc. Lond.* B. lxxii., 1903, p. 357; B. lxxiii., 1904, p. 128; B. lxxiv., 1905, pp. 147, 159; B. lxxvii. 1907, p. 211. Also in *Practitioner*, May 1908; various papers in *Lancet* and *Brit. Med. Journ.*

called by Wright and Douglas 'opsonin' (from a Greek word meaning 'to cater for').

They have also shown that during the process of active immunization the opsonic value of the serum is increased, and they have succeeded in demonstrating this opsonic immunity for a number of infections, such as the staphylococcic, Malta fever, pneumococcic, and tuberculous. If it be desired to measure the quantity of opsonins present, say in a case of furunculosis, which is almost always caused by the *M. pyogenes*, the following are required: (1) a drop or so of the patient's serum; (2) a drop of serum from a normal person; (3) a suspension of a culture of *M. pyogenes* in salt solution, preferably derived from the furuncle; (4) leucocytes washed free from the plasma. Equal volumes of the patient's serum, leucocytes, and suspension are mixed, drawn up into a capillary tube, incubated for fifteen minutes at 37° C., and films then prepared and stained. As a control a similar mixture is prepared and treated in the same way, but using the *normal* serum instead of that of the patient. The films are then examined, and the number of cocci taken up by, say, fifty leucocytes is counted in the two specimens, and a ratio obtained. Taking the figure for the normal serum as 1, that for the patient's serum will probably be 0.5 or 0.6, and this is termed the 'opsonic index' (see below, p. 192).

In subacute and chronic local infections the opsonic value of the serum is usually diminished, occasionally increased. In acute infections the index will, as a rule, be low; in chronic infections which are not strictly localized, e.g. tuberculosis, the index will sometimes be low, sometimes high. A low index generally indicates an infection, or a low power of resistance to the particular organism. A high index usually indicates that the person has had an infection but has overcome it. The normal index for healthy persons varies only within narrow limits, from about 0.8 to 1.2 as

extremes; an index above or below these values is therefore probably pathological.

By injecting small quantities of a vaccine consisting of a killed culture, tuberculin, &c., the opsonic index can be raised, and the infection thereby tends to be cured. The first effect of the injection is to cause a fall in the opsonic index, the 'negative phase' of Wright, which is usually afterwards followed by a rise, and by properly spacing the injections a considerable rise in the opsonic value may ultimately result. If too much vaccine be given the effect may be to permanently depress the index and cause harm instead of good, hence the desirability of controlling all injections by determinations of the opsonic index. By movement, massage, &c., applied at or about the seat of a local infection, bacterial products are disseminated which may alter the index; a process of auto-inoculation may thus result.

The opsonic index may be used for diagnostic purposes; a low or high opsonic value towards a particular organism suggests that an infection by this organism exists or has recently existed.

Bulloch came to the conclusion that the blood contains a number of specific opsonins, one for tubercle, another for *M. pyogenes*, and so on. Simon, Lamar, and Bispham,¹ however, from a number of carefully devised experiments conclude that specificity of opsonins does not exist, and suggest that opsonins may be a constant quantity, and that the number of organisms taken up by the leucocytes is influenced by a second unknown and variable factor.

Russell² also concludes that in *normal* serum the opsonins are 'common' and not specific, and can be removed by a number of bodies. In immune serum, on the other hand, both 'common' and 'immune' opsonins are present,

¹ *Journ. Exper. Med.* vol. viii., 1906, p. 651.

² *Johns Hopkins Hosp. Bull.* xviii., 1907, p. 252.

MANUAL OF BACTERIOLOGY

being quite specific. That is to say, in the immunization specific opsonins are formed, and the increase of opsonins following injection of a vaccine is due to the formation of immune opsonins which are specific.

Land and Martin¹ believe that in immune serum a thermolabile opsonin is present, and also a thermolabile opsonin.

Land considers the opsonins to be substances distinct from the antibodies, but Metchnikoff, Dean, and other observers believe that they are identical with the 'substance sensi-

ble to opsonic method has been criticised of late. Thus Moss² has shown that one of the present methods of estimating the opsonic index of the blood seems sufficiently accurate to be of practical value. Fitzgerald, Whiteman, and Strangeways,³ in an elaborate paper, concluded that the method is unreliable. Whereas in the present method the serum only, Shattock and Dudgeon⁴

3. A suspension of the organism for which the determination is to be made.

4. A suspension of living leucocytes.

5. Several Wright's pipettes with indiarubber teats or nipples.

1 and 2. *The Sera*.—These two specimens should be taken at about the same time, and the determination should be made as soon as possible, certainly within a few hours of taking the samples.

The blood is preferably collected in a Wright's capsule (fig. 34, *d*). Both ends of the pipette are broken off, and the

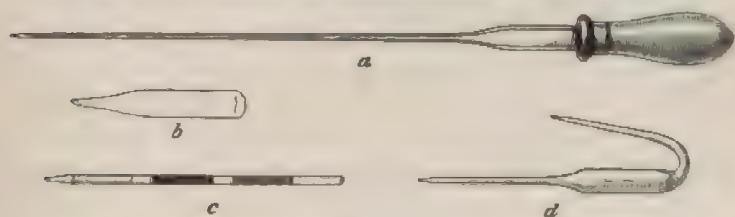


FIG. 34.

a. Glass pipette, with indiarubber teat for opsonic determinations, &c.; *b* shows (enlarged) the contracted extremity of the pipette; *c* shows the stem of the pipette, containing the equal volumes of serum, leucocytic suspension, and bacterial suspension, before mixing; *d* is the Wright's capsule for collecting blood.

blood is collected by immersing the *bent* end in the blood as it runs from a prick in the ear or finger. The capsule should be at least one-third filled. For pricking, a flat-pointed needle of the Hagedorn type is preferable; a prick with an ordinary needle does not yield sufficient blood. After filling, the capsule is sealed in the flame, the dry or straight end being sealed first. After coagulation the capsule is centrifugalized to obtain clear serum; for this purpose the capsule is hung by the curved end in the centrifuge. Little change in the serum ensues for two to three days if the capsules are kept sealed.

3. *Suspension of the Organism*.—In the case of tubercle,

MANUAL OF BACTERIOLOGY

dead cultures can be purchased. To prepare the emulsion from this, take a small portion (about as big as a pea) and place it in a small agate mortar and grind up with the pestle; then add 1.5 per cent. salt solution drop by drop until about 2 c.c. have been added, continuing to grind meanwhile. This gives an emulsion which contains isolated bacilli as well as clumps. These clumps must be got rid of, and to do this it is necessary to centrifugalize for three or four minutes. With the tubercle bacillus and gonococcus spontaneous phagocytosis is apt to occur if ordinary salt solution is used.

A phyllococcic emulsion is prepared by taking an emulsion not more than twenty-four hours old, adding a small amount of salt solution, and shaking gently so as to wash off the clumps. When the emulsion is made it must be pipetted into a small tube and centrifugalized for a few minutes. The emulsion must not be too thick, otherwise the leucocytes will take up an uncountable number of cocci; the

person is then pricked in the ear or finger, and his blood is allowed to drop into the fluid until 1 c.c. or more has been collected. The tube is then put into the centrifuge, very exactly counterbalanced, and centrifugalized until all the corpuscles have come to the bottom and the supernatant fluid is left clear. If the deposit is closely examined the red corpuscles will be seen to be at the bottom, whilst above them there is a thin whitish layer of leucocytes. Then, with a capillary pipette armed with an indiarubber nipple, or with a syringe, the whole of the clear fluid is pipetted off as close as possible to the leucocyte layer, but without disturbing the latter. The tube is then filled with saline solution, the blood and fluid are mixed, the mixture is centrifugalized, and the clear fluid again pipetted off, and this process of washing is repeated. Next, the leucocyte layer with the upper layer of red corpuscles (which also contains leucocytes) is pipetted off into a small tube, and the whole is thoroughly mixed by repeatedly sucking into, and expelling from, the pipette. The result is a suspension of living leucocytes mixed with red corpuscles.

5. *Wright's Pipettes with Indiarubber Teats*.—These are made of glass tubing drawn out in the blowpipe flame into the form shown in *a*, fig. 34, which is two-thirds full size. The end of the fine extremity should be contracted as shown in *b*. Glass tubing must be chosen which properly fits the teats.

The Process.—1. Prepare a pipette by placing an indiarubber teat on the thick end. Then, with a grease pencil or with pen and ink, make a transverse line about an inch from the pointed end. The volume of fluid contained in the tube between the point and this mark is spoken of as the unit.

2. Having the patient's serum and the suspensions of leucocytes and of bacteria ready to hand, take the pipette

between the index-finger and thumb of the right hand and compress the nipple. Immerse the point beneath the surface of the suspension of bacilli, and relax the pressure on the nipple until the emulsion has risen exactly to the mark so that one unit has been drawn up; then remove the point from the fluid and relax the pressure again so that a *small* volume of air is sucked up. This will be quite easy if the point is a good one, otherwise it will be difficult or impossible, as the column of fluid will either refuse to stir or will oscillate violently. Next immerse the point in the suspension of leucocytes and draw up one unit. This will be separated from the emulsion of bacteria by the bubble of air. Remove the point from the emulsion and draw up a second volume of air.

Lastly, draw up one unit of the serum. There will now be in the pipette (counting from the nipple towards the point) one unit of bacterial emulsion, a bubble of air, a unit of leucocytes, a bubble of air, and lastly a unit of serum (c, fig. 34).

3. Put the point of the pipette on to a clean hollow ground slide or an artist's porcelain sunk palette, and express the whole of its contents, and mix well together, aspirating them repeatedly into the pipette and expelling without causing bubbles. If bubbles form, a hot wire brought near will quickly dispel them. When thoroughly mixed, aspirate the mixture into the pipette, suck up a short volume of air, and seal the tip in the flame.

Then place the pipette point downwards in the incubator, or better into a water-bath at 35° to 37° C., noting the time exactly, and proceed to prepare a second pipette in precisely the same way, using the same suspensions of bacteria and leucocytes, but the control serum instead of the patient's. Place this in the incubator or water-bath, by the side of the other, noting the time at which this is done. When each

pipette has been incubated for a quarter of an hour it is removed from the incubator or water-bath, the end broken off and the nipple fitted to the thick end, then the contents are expelled on to a hollow slide or porcelain palette and mixed thoroughly together. Films are then prepared. This may be done by depositing a drop in the middle of a large cover-glass (1-inch squares, No. 2), dropping on to it another cover-glass and drawing the two apart. Or the films may be made on slides, for which Wright recommends roughing the slides with emery paper and spreading the drop with the sharp edge of a slide broken in half after scratching the side with a file or glass knife. The films then have to be stained. For staphylococci, streptococci, pneumococci, *B. coli*, &c., the films may be fixed with formalin and stained with carbol-thionine blue, or they may be stained without previous fixing with the Leishman stain. For tubercle, the films may be fixed with mercuric chloride solution (one to two minutes), stained in warm carbol fuchsin, decolorized with 2½ per cent. sulphuric acid in methylated spirit, and counter-stained with methylene blue.

Lastly, the films are examined with the oil-immersion lens, preferably with the aid of a mechanical stage, and the number of organisms contained in not less than fifty polymorphonuclear leucocytes counted. Parts of the film in which the cells are broken down or not well stained, or cells containing obvious clumps of organisms should be avoided. The ratio between the number in the control and the number in the specimen prepared with the patient's serum gives the *opsonic index*. Thus, if in the control there are 125, while in the patient's specimen there are 75, the index would be $\frac{75}{125} = 0.6$, i.e. not much more than half the normal.

Preparation of Vaccines for Treatment &c.—The vaccine used for treatment is a sterilized, standardized suspension of the infecting organism, except in the case of tuberculosis, for which

the new tuberculin (TR) or an analogous preparation is employed. In certain instances a mixture of organisms is used—e.g. *M. pyogenes*, var. *aureus* and var. *albus*, in some cases of acne—and the strain of organism isolated from the lesion is often to be preferred. The general principle of preparation of the vaccine is to suspend a recent surface agar culture of the organism in 4–5 c.c. of sterile 1 per cent. salt solution, to heat this suspension to 60° C. or thereabouts for half an hour, to make subcultures from the heated suspension to control its sterility, and to standardize the suspension so that it contains a given number of organisms in 1 c.c. The lower the temperature to which the suspension is heated *consistent with sterilization*, the more active the vaccine. The standardization is usually performed by Wright's method,¹ which may also be controlled by plating the diluted suspension *before* heating, and by ascertaining by weighing the amount of dry residue in a given volume of the suspension. In Wright's method a volume of normal blood of a male is mixed with 1 volume of the suspension by means of a Wright pipette (fig. 34, a), and this mixture may be further diluted with 1–5 volumes of salt solution if necessary, and the whole is thoroughly mixed. Films of the mixture are then made on slides, the films stained with Leishman's stain, and the relative numbers of red corpuscles and of bacteria in the film counted, in a number of fields, preferably by the use of a high-power eyepiece ruled with cross lines. Assuming that there are 5,000,000 red corpuscles per cubic millimetre of blood, the number of bacteria per cubic centimetre of suspension is readily calculated.² Thus, if equal volumes of blood and suspension are mixed and the number of red corpuscles and of bacteria are approximately equal, the suspension evidently contains 5,000,000 organisms per cubic millimetre, or 5,000,000,000 per cubic centimetre. A staphylococcic suspension of an agar culture in 10 c.c. of salt solution will probably contain about 1500–3000 million organisms per c.c. It is necessary in all cases before administering the vaccine to make a count of the number of organisms it contains

¹ *Lancet*, July 5, 1902.

² One cubic centimetre contains 1000 cubic millimetres.

The following table¹ gives an idea of the doses of vaccines, their toxicity, and frequency of inoculation :

Vaccine	Relative Toxicity	Doses	Frequency of Inoculation
Tuberculin	Very toxic	1000-4000 mgrm.	Every 10-14 days
<i>B. coli</i>	Very toxic	5-15 millions	Every 2, 5, or 10 days
Pneumococcic	Less toxic than <i>B. coli</i>	10-50 millions	Every 36-48 hours in pneumonia; every 10 days in chronic infections
Streptococcic	More toxic than pneumococcic	20-60 millions	Every 7-14 days
Staphylococcic	Less toxic than streptococcic	100-1000 millions	Every 10 days
<i>M. melitensis</i>	--	1 ¹ / ₂ sq. cm. of surface agar culture (because very difficult to count)	Every 7-14 days
Gonococcic	Slightly toxic	100-500 millions	Every 7-14 days

The smaller doses are given at the commencement of the treatment, and the doses are gradually increased. The effects of the treatment and regulation of dosage are controlled by opsonic determinations, at least in the earlier stages, and at intervals later.

ADDENDUM.

ANAPHYLAXIS is the term applied to the property which certain toxins possess of increasing, instead of diminishing, the susceptibility of an organism to their action, the result being that after an animal has received a first dose with impunity, the administration of a second dose, no larger in amount, produces severe intoxication. This may occur with toxins, serum, etc. (see 'Supersensitisation,' p. 149).

BACTERIOTROPINES.—This name was given by Neufeld and Rimpau to substances found in the serum of artificially immunized animals which acted upon bacteria in such a way as to promote phagocytosis. They may be identical with opsonins.

HAPTINES.—Ehrlich has termed the diverse free receptors, cast off into the body fluids in various circumstances, 'haptines,' e.g. antitoxin.

¹ See Harris, *Practitioner*, May 1908, p. 647.

CHAPTER VI.

SUPPURATION AND SEPTIC CONDITIONS.

The subjects of septic infection and of suppuration are of great practical importance, and a knowledge of their ætiology is one of the main factors which have conduced to the great advances that were made during the Victorian era in the treatment of wounds, whether accidental or made by the surgeon's knife.

Ogston in 1881 and Rosenbach in 1884 demonstrated that micro-organisms are almost invariably present in the pus of acute abscesses, and these observations were repeatedly confirmed by subsequent investigators. A number of experiments were then initiated in order to ascertain whether these organisms bear a causal relation to the phenomena of suppuration or are merely accidentally present. These experiments showed that a large number of organisms can produce suppuration, and render it certain that in ninety-nine cases out of a hundred the suppurative and septic conditions met with spontaneously, or occurring after surgical interference, are due to the action of micro-organisms. The chief of these are several micrococci (commonly known as *staphylococci*, and the infections which they produce, as *staphylococcic infections*) and streptococci.

Under the terms suppuration and septic diseases are included such varied conditions as abscesses, boils and carbuncles, cellulitis, osteomyelitis, erysipelas, gonorrhœa,

infective endocarditis, pyæmia, septicæmia and sapræmia, puerperal fever, and hospital gangrene.

As will be gathered from the descriptions of the individual organisms, suppuration may be set up by inoculation with several species, and a number of experiments by various observers, carried out by inunction, subcutaneous inoculation, and inoculation into the serous cavities and circulation, have conclusively proved that this is the case, not only in animals, but also in man.

A problem of great importance is whether micro-organisms are usually the cause of suppuration, or whether mechanical injury, chemical agents, &c. can also produce it. Mechanical injury alone does not seem to be capable of inducing pus production, but it is otherwise with regard to chemical agents. For a long time considerable difference of opinion existed and discordant results were published. These discrepancies have now been cleared up, and are found to depend upon the method of experiment and the particular animal and chemical agent employed. That chemical agents should produce suppuration was only to be expected, for it would be against analogy, derived from all other bacterial diseases, if the pyogenic organisms do not produce suppuration through the chemical substances formed by, or present within, their cells, and if these chemical substances act thus, why should not other chemical substances be found to act in a similar way?

In experiments with chemical agents the greatest care has to be taken to exclude the entrance of micro-organisms. This is best done by sealing the sterilized substance in sterilized fusiform glass tubes and introducing these under the skin or into the tissues with strict aseptic precautions. When the wounds have completely healed the tubes are broken by pressure and their contents allowed to diffuse into the surrounding tissues.

Sterilized cultures (above a certain amount) of the

Micrococcus pyogenes and a crystalline body, phlogosin, obtained by Leber from its cultures, produce abscesses on inoculation. Mercury produces suppuration in the dog, but not in the rabbit; silver nitrate (5 per cent. solution) has a similar action. Ammonia fails to produce pus; it is either absorbed without damage, or if in stronger solution produces necrosis of the tissues. Turpentine produces large sterile abscesses in carnivora, and Brieger's cadaverine is likewise stated to set up suppuration.

Buchner was also able, by warming various bacteria with 0.5 per cent. caustic potash, to obtain a solution containing protein which was powerfully pyogenic, and Nannotti found that sterilised pus had a similar property. It thus seems certain that a number of chemical substances can set up suppuration. At the same time, it must be clearly recognized that suppuration and suppurative complications, as they occur naturally, are to be regarded as due to the activity of micro-organisms in almost every instance.

Of so-called 'septic' diseases, sapræmia, septicæmia, and pyæmia must be mentioned. By 'sapræmia' is meant the constitutional condition arising from the absorption of the toxic products elaborated by micro-organisms, the latter being localized and absent from the general circulation. In the acute form it is not a common condition, the best example being that which occurs after parturition; by simply clearing and washing out the uterus the symptoms rapidly abate. In septicæmia not only is there usually (though not necessarily) a local site of infection, but in addition micro-organisms are present in the general circulation. It is true they are not abundant in the latter situation, and Cheyne¹ believes that they are to a large extent arrested in the capillaries. Micrococci and streptococci are the commonest forms. Pyæmia is characterized by the presence of micro-organisms, most frequently

¹ *System of Medicine*, Clifford Allbutt, Ed. 2, i. p. 876.

streptococci, in the general circulation and in addition by the formation of abscesses in various situations. These arise usually from suppurative phlebitis with the formation of septic emboli and thrombi. The sequence of events, according to Cheyne (*loc. cit.* p. 881), is (a) phlebitis in direct connexion with the wound, (b) a thrombus impregnated with micro-organisms is formed in the vein, (c) this softens and disintegrates, and particles or emboli are carried to distant parts, (d) these lodge in the capillaries with the formation of infarctions and abscesses. Suppurative pyle-phlebitis is a pyæmia affecting the portal system of vessels. As regards the so-called chronic pyæmia or multiple abscesses, Cheyne considers that it differs from true pyæmia in that embolism plays no part. Organisms gain access to the blood-stream, settle in any spot where the vitality of the tissues is depressed, grow and multiply, and there produce an abscess.

The mere presence of micro-organisms does not always suffice, however, for they may be present without producing suppuration; and the same organism, for example the *Streptococcus pyogenes*, may at one time produce a localized abscess, at another diffuse cellulitis, and at a third pyæmia; a number of conditions and factors control and modify the occurrence and the particular form of septic disease.

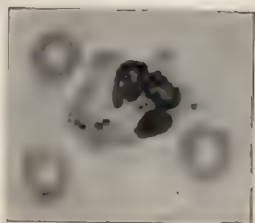
As already alluded to (p. 179), many micro-organisms when injected into the blood-stream are rapidly disposed of; so when moderate quantities of the *Micrococcus pyogenes* are injected into the circulation of a rabbit, abscesses as a rule form only in the kidney. If, however, the organisms be attached to gross particles, so that they cannot pass through the capillaries, embolism occurs and abscesses form about the embolic foci. The effect of i and injury in making a part 'susceptible' marked. Inject the *M. pyogenes* into anim

endocardium or a bone has been damaged, and in all probability an endocarditis or osteomyelitis will ensue. The dose and concentration of the organisms are also important factors. Watson Cheyne found that 250,000,000 cocci (*M. pyogenes*) injected into the muscles of a rabbit, produced a circumscribed abscess, but 1,000,000,000 caused death. So, probably, while the cells in a healthy wound can dispose of a few organisms, if the latter are abundant or in masses they will gain the mastery.

MICROCOCCUS PYOGENES, VAR. *AUREUS* (STAPHYLOCOCCUS
PYOGENES AUREUS).

Morphology and Biology.—A minute spherical organism measuring about $0.75\ \mu$ in diameter. It occurs generally in more or less irregular groups, but may be met with singly or in pairs (Plate I., c). It is non-motile, does not form spores, and stains well with all the anilin dyes and also by Gram's method. It is aerobic and facultatively anaerobic, and will grow *in vacuo*. It grows well and rapidly on all the usual culture media at temperatures from 18° to 37° C. On agar-agar it forms a thickish, moist, shining growth, cream-coloured at first, but after a day or two developing a characteristic orange-yellow colour. It grows in the same manner on blood serum without liquefaction of the medium. Gelatin is rapidly liquefied, the liquefied gelatin being at first somewhat turbid from yellowish masses of organisms; these later on subside and form an orange-yellow sediment (Plate I., d). In gelatin plates the colonies form at first small whitish, granular points, developing in two or three days into circular areas of liquefaction with yellowish masses of the organism floating in them. On potato it forms a growth similar to that on agar. When grown in milk it produces coagulation. Acid production (lactic and butyric acids) can be demonstrated by growing on a neutral litmus glucose-agar. When grown

PLATE I.

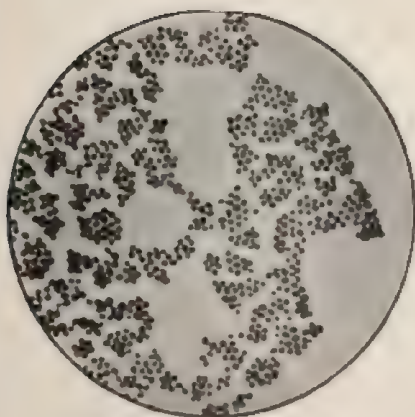


a.

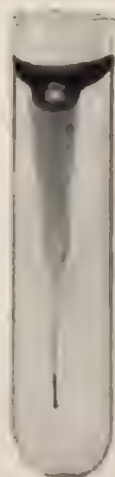


b.

PHAGOCYTOSIS BY POLYMORPHONUCLEAR LEUCOCYTES.
a. *M. PYOGENES*, VAR. *AUREUS*. b. *B. TUBERCULOSIS*



c. *M. PYOGENES*, VAR. *AUREUS*.
COVER-GLASS PREPARATION OF A PURE
CULTURE. $\times 1500$.



d. *M. PYOGENES*, VAR. *AUREUS*.
GELATIN STAB-CULTURE,
4 DAYS OLD.



in broth or peptone water it gives the indole reaction with the addition of a nitrite, but not without.

The rate of liquefaction of gelatin and the pigment production vary; the latter is sometimes much deeper than at others, recently isolated cultures show it better than old ones, and the presence of oxygen seems to be also necessary (see p. 94). The amount of acid production appears to vary directly with the virulence, which is likewise very variable.

Pathogenicity.—The *Micrococcus pyogenes*, var. *aureus*, is by far the commonest of all organisms met with in suppurative processes. Ogston found it alone in thirty-four, and associated with the *Streptococcus pyogenes* in sixteen, out of sixty-four cases of abscess. It occurs in acute abscesses, boils, and acne, in some cases of puerperal fever and infective endocarditis, and is almost invariably found in osteomyelitis, but only occasionally in pyæmia. The organism injected under the skin of man or animals produces an abscess, and injection into the blood-stream under certain conditions is followed by infective endocarditis or pyæmia. Impetigo pustules are produced by inoculation into the skin.

It may be said to be universally present on all parts of the skin, and in the mouth, and is frequently met with in the air. According to Sternberg, recent cultures in gelatin are destroyed by an exposure to a temperature of 56° to 58° C. for ten minutes; but when dried much higher temperatures, 90° to 100° C., are required, and in the dried state (on a cover-glass) it retains its vitality for more than ten days. According to different experimenters, from five to fifteen minutes are required to destroy it with a 1-1000 mercuric chloride solution; but it is evident that much depends on the state of aggregation of the organisms, and Abbott has shown that while most of the cocci in a culture are destroyed in five minutes, a few may survive much longer.

Toxins.—In a case of infective endocarditis examined

by Sidney Martin,¹ due to the *M. pyogenes*, var. *aureus*, a large amount of an albumose and of a basic body was extracted from the blood and spleen. The albumose produced fever and wasting, and retarded the coagulation of the blood.

Leber extracted a crystalline body, which he termed phlogosin, from cultures of the *M. pyogenes*, var. *aureus*, and Brieger also obtained a crystalline base.

The decomposition products of the action of the *M. pyogenes*, var. *aureus*, on egg-albumin are, according to Emmerling,² phenol, indole, and skatole, many volatile and non-volatile acids, betaine, and trimethylamine.

Anti-Serum.—Attempts have been made to prepare an anti-serum by the injection of cultures, but the serum is of no practical value. A *vaccine*, prepared by heating a suspension of an agar culture to 65° C. for half an hour and standardizing, has been used with much success in chronic staphylococcic infections, such as acne and boils.

MICROCOCOCCUS PYOGENES, VAR. ALBUS, AND VAR. CITREUS.

MICROCOCOCCUS EPIDERMIDIS. MICROCOCOCCUS CEREUS.

These organisms are of rarer occurrence than the preceding one. In morphology and cultural characteristics the first two agree with the *Micrococcus pyogenes*, var. *aureus*, except that the *albus* produces a white, shining, porcelain-like growth, and the *citreus* a lemon-yellow growth, on agar. They are said to be less pathogenic than the *aureus*, and are only occasionally found alone, being usually associated with the *aureus*. Cheyne, however, states that in his experience the *albus* is more virulent than the *aureus*, and mixed infections with the *aureus* are regarded as more severe than infection with the *aureus* alone. The *albus* has been found in some cases of panophthalmitis, and is said

¹ 'Chemical Pathology of Diphtheria,' &c., Gulstonian Lectures, *Brit Med. Journ.* 1892, i. p. 755.

² *Ber. der Deutsch. Chem. Gesellsch.* 1896, 29, p. 2721.

by Flügge to be commoner than the *aureus* in the lower animals.

Andrewes and Gordon¹ regard the *aureus*, *albus*, and *citreus* merely as variants of a single species, the *Micrococcus pyogenes*. They found that every variety of colour, from orange, through yellow to white, might be obtained by cultivation. The *Micrococcus flavescens*, met with by Babes in abscesses, must probably be placed in the same category. On the other hand, the *Micrococcus epidermidis* (*albus*), first described by Welch as occurring on the skin, in stitch abscesses &c., and feebly pathogenic compared with the *M. aureus*, is stated by these authors to be perfectly distinct from the foregoing. Other organisms which are occasionally met with in abscesses, the *Staphylococcus cereus albus* and *S. cereus flavus* of Passet, form shining waxy growths on agar, and do not liquefy gelatin, and are probably variants of another species, which may be termed the *Micrococcus cereus*. There may be many other varieties of micrococci not yet properly differentiated.² Well-defined micrococci occur in the saliva (*M. salivarius*), and in the scurf from the scalp. Andrewes and Gordon give the following differential table of some of these micrococci :

Chief Types of Human Micrococci.

Organism	Broth Culture	Pigment on Agar	Clot in Milk	Liquefaction of Gelatin	Reduction of Neutral Red	Reduction of Nitrate	Acid formation from				Pathogenicity
							Maltose	Lactose	Glycerin	Mannitol	
<i>Micrococcus pyogenes</i>	Turbid	Orange, yellow, or white	+	+	0	+	+	+	+	+	+
<i>Micrococcus epidermidis</i>	Turbid	White	+	+	+	+	+	+	+	0	Feeble
<i>Micrococcus salivarius</i>	Clear	White	0	0	0	+	+	0	+	0	0
Scurf Micrococcus	Turbid or clear	White	0	0	0	+	0	0	0	+	0

¹ Rep. Med. Off. Loc. Gov. Board for 1905-06, p. 543.

See Gordon, Rep. Med. Off. Loc. Gov. Board for 1903-04, p. 388.

MICROCOCCLUS ZYMOGENES.

Isolated by MacCallum and Hastings¹ from a case of acute endocarditis. A minute micrococcus, non-motile, and staining by Gram's method. On surface agar it forms a thin, slightly elevated, moist, glistening, greyish-white growth. In gelatin stab-cultures the growth is somewhat opaque and granular, with slow liquefaction. Blood serum is slowly liquefied. On potato a thick, moist, dirty-white growth develops, becoming dry and brownish after three days. Broth becomes slightly clouded after twenty-four hours' growth, but in three to four days the organisms settle to the bottom, leaving the medium clear. No indole and no gas are formed. In neutral litmus milk, the litmus is decolorized after a few hours, and in twenty-four hours the milk is firmly curdled. Somewhat later liquefaction of the curd ensues from above downwards; at first the turbid fluid is reddish in the superficial layer and yellowish below, ultimately the whole curd is transformed into a turbid liquid with a reddish colour throughout. These changes in milk are characteristic of the organism. It is pathogenic to white mice, hardly so to guinea-pigs and white rats, and moderately so to rabbits, by intravenous inoculation into which endocarditis is sometimes produced. Harris and Longcope² have reported five more instances of the occurrence of this organism (once from a cesspool, four times as secondary invasions at autopsies), and Birge³ has isolated a similar but less virulent organism from the larynx of crows.

MICROCOCCLUS NEOFORMANS.

This organism was isolated by Doyen from malignant growths, and was supposed by him to be the causative organism of malignant disease. It is a typical Gram-positive coccus, giving a white growth on agar and liquefying gelatin in three to four days. According to Dudgeon and Dunkley,⁴ it gives all Gordon's fermentation tests for the *M. pyogenes*, var. *albus*, except that it does not acidify mannitol.

¹ *Journ. Exp. Med.* iv. 1899, p. 521.

² *Centr. f. Bakt.* (1^{te} Abt.), vol. xxx., 1901, p. 353.

³ *Johns Hopkins Hosp. Bull.* xvi., 1905, p. 309.

⁴ *Journ. of Hygiene*, vii., 1907, p. 13.

The serum of patients suffering from malignant disease does not give any marked agglutination with the *M. neoformans*, nor does it contain opsonins specific for the organism. The *M. neoformans* is non-pathogenic for rats and mice.

STREPTOCOCCUS PYOGENES.

Next to the *Micrococcus pyogenes*, this organism is of the greatest practical importance.

Morphology.—A non-motile, spherical coccus measuring about $1\ \mu$ in diameter. It stains well with anilin dyes and is Gram-positive.

Multiplication is by fission in one direction only, so that chains of cocci are formed. In pus these chains do not usually contain more than ten to fifteen elements (Plate II., *a*), but under cultivation, and especially in broth cultures, they may be much longer, and occasionally in a chain an irregular division occurs, so that a branch chain forms. A cell here and there in a chain is often somewhat larger than its fellows, and some authors have considered these enlarged individuals to be arthrospores (Plate II., *b*).

Cultural Reactions.—It can be cultivated on the usual culture media, and grows anaerobically as well as aerobically. On agar, or better glycerin agar, it forms in twenty-four to forty-eight hours minute whitish, semi-transparent, more or less isolated colonies (Plate II., *c*). On gelatin the growth has much the same characters, and is better seen, as this medium is clearer than agar, but it takes some days to attain the maximum. In stab-cultures it forms minute spherical colonies all down the line of the stab, but does not invade the surrounding medium; the gelatin is not liquefied. In broth it forms a flocculent deposit, the fluid generally remaining clear. It does not develop on potato, but in milk it grows well, usually, but not always,

with coagulation. The indole reaction can be obtained in broth cultures in seven to fourteen days on the addition of a nitrite, but not without. It gives an acid reaction when grown on neutral litmus glucose-agar. It is the only organism with which the writer is acquainted that does not reduce a weak solution of methylene blue.

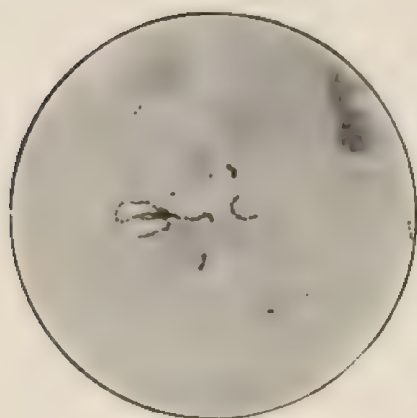
According to Sternberg, the thermal death-point of *Streptococcus pyogenes* is 52° to 54° C., the time of exposure being ten minutes. It is destroyed by a two hours' exposure to 1-300 carbolic acid, and 1-2400 mercuric chloride solutions.

The *Streptococcus pyogenes* is sometimes found in acute circumscribed abscesses, in 16 per cent. according to Zuckermann from an analysis of the results of various observers. The streptococcus is, however, especially frequent in spreading inflammations, lymphangitis, cellulitis, and progressive gangrene, and is the usual cause of pyæmia and puerperal fever. It is met with in about one-third of the cases of infective endocarditis, occasionally in acute osteomyelitis, and seems to be the cause of the septic pneumonia so often observed after operations about the mouth and throat.

In erysipelas, streptococci are present in the lymphatics at the margin of the zone of redness. These were first isolated by Fehleisen, who described the organism as the *Streptococcus erysipelatis*, and by inoculation experiments on man and animals demonstrated its causal relation to the disease. The experiments on man were made in cases of extensive and inoperable carcinoma and sarcoma, as it had been noticed that malignant tumours were frequently benefited after an attack of erysipelas. Several cases were inoculated, and all were successful with one exception, typical erysipelas developing (see Coley's Fluid, p. 227). Jordan,¹ however, has found experimentally that typical

¹ *Munch. Med. Woch.* Aug. 27, 1901.

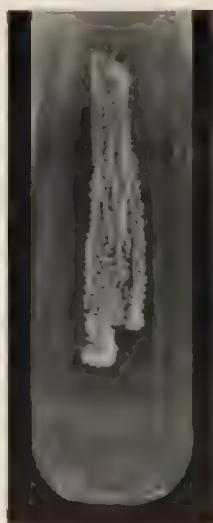
PLATE II.



a. STREPTOCOCCUS PYOGENES IN PUR.
SMEAR PREPARATION. $\times 1000$.



b. STREPTOCOCCUS PYOGENES.
COVER-GLASS PREPARATION OF A BROTH
CULTURE. $\times 1500$.



c. STREPTOCOCCUS PYOGENES.
PURE CULTURE ON
GLYCERIN AGAR.



erysipelas may be produced in a rabbit's ear not only with the streptococcus, but also with staphylococci, pneumococci, and *B. coli*, and he believes that although streptococci usually cause human erysipelas, this disease may also be produced by staphylococci, and possibly by the pneumococcus, *B. coli*, and even the *B. typhosus*.

At one time the *Streptococcus erysipelatis* was considered to be different from the *Streptococcus pyogenes*, but the two organisms are now regarded as identical, the differences in cultural characters being slight and not constant. Petruschky¹ produced a typical erysipelas in the human subject by inoculation with a pure culture of a streptococcus derived from a case of suppurative peritonitis, and Bulloch² has shown that an animal immunized against a streptococcus derived from a case of erysipelas is also immune against a streptococcus isolated from an abscess (but see below, p. 213).

The different effects produced by the *Streptococcus pyogenes*, abscess in one case, erysipelas in another, cellulitis or pyæmia in a third, are attributable partly to differences in virulence, partly to the seat of infection and mode of entrance into the body, partly to real differences existing between different races of streptococci (see below, p. 212). Streptococci have been described in a number of diseases about which we know little, such as variola, scarlatina (*S. scarlatina* or *conglomeratus*), and vaccinia, but it is uncertain what causal relation they bear to these conditions. Strangles, a disease of horses, seems to be due to streptococci.

Anti-Serum.—The important lesions due to the streptococcus and their grave nature have led to the attempt to prepare an anti-serum, but many and great experimental difficulties have to be overcome to do this. The virulence

¹ *Zeitsch. f. Hyg.* xxiii., 1896, p. 142.

² *Trans. Brit. Inst. of Prev. Med.* i., 1897, p. 6.

of the streptococcus has to be increased by passing it through a series of rabbits, and it is only by growing it in serum media that satisfactory cultures for the inoculation of the horses can be prepared. Human serum is the best, but is difficult to obtain; a mixture of asses' serum and peptone beef-broth comes next. The cultures are grown for about a fortnight and are then inoculated into horses, first killed and then living cultures being used, and after a time the blood acquires anti-microbial properties. It is customary now to make use of a 'polyvalent' serum, i.e. one prepared by the injection of many strains of streptococci. The streptococcus anti-serum has been employed in erysipelas, cellulitis, puerperal fever, and pyæmia, in many cases with apparent success. Cheyne also suggests its use as a prophylactic before operations about the mouth and throat to avoid septic pneumonia.

A vaccine prepared by sterilizing cultures with heat may be used with benefit in subacute and chronic streptococcal infections.

There are slight differences in the cultural characters and morphology of the streptococci derived from different sources, and the virulence varies very considerably. Von Lingelsheim has named two varieties, *brevis* and *longus*, the former rendering broth turbid, growing in short chains, and being non-pathogenic to mice and rabbits, the latter leaving the broth clear, growing in long chains, and always pathogenic to these animals. By some these are considered to be merely accidental varieties and not true species.

Gordon¹ divided the streptococci into four varieties, viz. (1) the *S. longus*, restricted to an organism forming exceptionally long chains; (2) *S. medius*, including the majority of streptococci from pus, sepsis, and erysipelas, and Lingelsheim's *longus*; (3) *S. brevis*, including Lingelsheim's *brevis* and the *Diplococcus pneumoniae*; (4) *S. scarlatinae* or *conglomeratus*. In addition to

¹ Rep. Med. Off. Loc. Gov. Board for 1898-99, p. 482.

differences between the colonies on agar and gelatin, the following table illustrates other differences :

	<i>S. longus</i> . Isolated from the mouth	<i>S. medius</i> . From pus &c.	<i>S. brevis</i> * & Lingelsheim's variety ; also <i>Dip. pneumoniae</i>	<i>S. scarlatinae</i> or <i>conglomeratus</i>
Morphology	Very long and comparatively straight chains.	Medium-sized, curling, chains.	Short chains of only a few elements.	Masses of chains. Apparently bacillar (diphtheria-like) forms appear under certain conditions.
Broth	Remains clear. Flocculent masses of growth form at bottom of tube with no coherency.	Same as <i>S. longus</i> .	Becomes turbid throughout.	Remains clear. Growth forms white masses with marked tendency to retain their coherency.
Gelatin at 37° C.	Similar to broth.	Similar to broth.	Becomes uniformly turbid.	Similar to broth.
Litmus Milk	Growth confined to the bottom of the tube. Acid reaction. No curdling.	Acid production slight. No curdling.	Slight acid production. Milk usually curdled.	Much acid production. Marked curdling.

* Washbourn and Goadby state that the *S. brevis* is the commonest species in the mouth.

Recently, considerable attention has been directed to the differentiation of streptococci by Houston,¹ Andrewes,² Andrewes and Horder,³ Gordon,⁴ and Besredka.⁵ By cultivating in media containing various sugars, alcohols, glucosides, &c., considerable differences are found in the biological properties of various strains of streptococci. Andrewes and Horder distinguish (1) *Streptococcus pyogenes*, from pus, erysipelas, cellulitis, pyæmia and septicæmia, endocarditis, &c. (2) *S. salivarius*, the common type in the saliva. Also met with, probably as a 'terminal' infection, in endocarditis and septicæmia. Shades into the *S. faecalis* and *S. anginosus*. (3) *S. anginosus*, from inflamed and scarlatina throats, endocarditis, and rheumatism. (4) *S. faecalis*, abundant in fæces, air, and dust. Met with also in endocarditis, meningitis, cystitis, and suppuration. Two strains of the *Diplococcus*

¹ Rep. Med. Off. Loc. Gov. Board for 1902-03, p. 511, and 1903-4, p. 472.

² Lancet, Nov. 24, 1906.

³ Ibid. 1906, ii. Sept.

⁴ Ibid. Nov. 11, 1905, and Rep. Med. Off. Loc. Gov. Board for 1903-04.

⁵ Bull. de l'Inst. Pasteur, ii. 1904, Nos. 16 and 17.

rheumaticus proved to be this organism. (5) The pneumococcus. (6) *S. equinus*, present in the intestine of herbivora. They remark that they do not assert that these are absolutely defined species; at the most they seem to be species in the making, and are connected by transitional forms.

Andrewes and Horder give the following table summarising the characters of the various streptococci:

Name	Milk Clot	Reduction of Neutral Red	Cane Sugar	Lactose	Inulinose	Inulin	Gelatin	Conferin	Mannitol	Growth on Gelatin at 20° C.	Morphology	Pathogenicity to Mouse	Hemolysis
<i>Streptococcus pyogenes</i>	-	-	+	+	-	-	±	-	-	+	longus	+	+
<i>Streptococcus antisepticus</i>	+	±	+	+	±	-	-	-	-	±	brevis	-	0
<i>Streptococcus anginosus</i>	+	±	+	+	-	-	-	-	-	±	longus	+	+
<i>Streptococcus faecalis</i>	+	+	+	+	-	-	+	+	+	+	brevis	-	0
<i>Streptococcus equinus</i>	-	-	+	+	-	-	+	+	+	+	brevis	-	0
<i>Streptococcus pneumoniae</i> (Pneumococcus)	±	-	+	+	+	±	-	-	-	+	brevis	+	0

+ = Positive or acid-production.

- = Negative or no acid-production.

± = Acid-production sometimes present, sometimes absent.

(These differences are not constant; with various strains one or other reaction may be lacking.)

BACILLUS PYOCYANEUS.

This is the organism found in green and blue pus, and it also occurs on the surface of the body. Its presence in wounds greatly retards healing, and occasionally a general toxæmia may result from it. It has been met with in otitis media and in the green pus of the pleural and pericardial cavities. It is a slender bacillus measuring 3 to 4 μ , frequently united in pairs and forming filaments. It is actively motile, does not form spores, and is aerobic and facultatively anaerobic. It does not stain by Gram's method. On gelatin it grows freely with rapid liquefaction, a greenish, fluorescent colour developing in the liquid, while whitish flocculi of growth sink to the bottom. On agar a whitish,

moist layer develops, and the medium is stained a greenish colour. On potato the growth is a dirty brown or sometimes greenish.

Milk is coagulated and a greenish colour develops. Broth becomes turbid, and there is a slight film formation with a greenish colour. Oxygen is necessary for the development of the pigment, which is generally a mixture of a blue pigment, pyocyanin, and a yellow one, pyoxanthose. Pyocyanin ($C_{14}H_{14}N_2O$) is said to be an anthracine derivative; it is soluble in chloroform, and on oxidation yields pyoxanthose.¹

Subcutaneous inoculations of a small amount of a culture produce local abscesses, larger amounts cause œdema with purulent infiltration of the tissues and death. Animals can be vaccinated by means of small quantities of living cultures or by sterilized cultures. Sterilized cultures will prevent infection (experimentally) by anthrax if used early—that is to say, if an animal be inoculated with anthrax, and shortly afterwards injected with a broth culture of the *Bacillus pyocyaneus*, a fatal result is averted. Emmerich and Loew² claim to have isolated from cultures a ferment-like body, 'pyocyanase,' which they state has preventive and curative properties towards anthrax and diphtheria infections. Emmerich³ has employed the dry pyocyanase as an application in diphtheria to dissolve the false membrane.

Williams and Cameron⁴ describe four cases of diarrhoea with green stools, wasting, and death, in infants, in which the *B. pyocyaneus* was obtained, and suggest that many cases of marasmus may be due to it. A form of epidemic dysentery seems occasionally to be caused by this organism (see Dysentery). A few cases of general infection with

¹ See *Centr. f. Bakt.* xxv. p. 897; *Journ. Exp. Med.* Sep.-Nov. 1899.

² *Zeitschr. f. Hyg.* 1899; *Centr. f. Bakt.* xxxi. (Originale) p. 1.

³ *Münch. Med. Woch.* Nov. 5 and 12, 1907.

⁴ *Journ. Path. and Bact.* iii., 1896. p. 344. (Refs.)

this organism have also been recorded. It has also been isolated from conditions of dermatitis and bullous eruptions.¹ The *B. pyocyaneus* has been found in water, dung, soil, and in the effluent from filter beds. Lehmann and Neumann state that, with the exception of pathogenicity, there is no essential difference between this organism and the *B. fluorescens liquefaciens* so frequently met with in water.

Various races of the *B. pyocyaneus* exist, differing in their pigment production.²

CLINICAL EXAMINATION.

In many cases some idea can probably be formed as to the organisms likely to be present in the pus or discharge &c. from the clinical characters of the disease, in which case the examination may be more particularly directed towards the isolation of the suspected organism. For example, in a urethral discharge the gonococcus would be specially looked for, in an empyema following pneumonia the *Diplococcus pneumoniae*, and in a tropical abscess of the liver the *Amoeba coli*. In all cases the pus or discharge should be collected with aseptic precautions in sterile capillary pipettes or in sterile test-tubes at the time of operation. The discharge from opened abscesses and from wounds is liable to become contaminated and the original infection to be masked. In septic wounds the infection may be a mixed one.

In all cases the examination should be commenced as early as possible.

1. Make several smears from the pus or discharge.

2. Stain one or two of these with Löffler's blue and one or two by Gram's method. Mount and examine microscopically.

(a) If staphylococci only are detected, the presence of the ordinary pyogenic cocci may be suspected. Proceed as in 3, 4, and 5.

(b) If encapsuled diplococci are detected, suspect the presence of the *Diplococcus pneumoniae*, and proceed as in 3, 5 and 7.

¹ See Pernet, *Brit. Med. Journ.* 1904, ii, p. 992.

² *Ann. de l'Inst. Pasteur*, v., 1891, p. 65.

(c) If diplococci and tetrads are present, note whether they are in groups within the pus cells ; if so, suspect the presence of either the gonococcus or *Diplococcus intracellularis meningitidis*, and proceed as in 6.

(d) If free tetrads are detected, suspect the presence of the *Micrococcus tetragenus*, and proceed as in 8 and 4 (rare).

(e) If streptococci are present the *Streptococcus pyogenes* is probably the species. Proceed as in 8, 4, and 5.

(f) If bacilli are present they may be the colon bacillus, the *Bacillus Welchii* (*aerogenes capsulatus*), the bacillus of malignant œdema, the tetanus bacillus, the typhoid bacillus, the *Bacillus pyocyaneus*, or putrefactive bacilli of the *Proteus* group (which see). The result of Gram-staining and the clinical history of the case will be some guide.

a. The colon bacillus, especially frequent in suppurative peritonitis and in diseases of the urinary organs. See page 363.

β. The *Bacillus Welchii* (*aerogenes capsulatus*), especially met with in foul wounds and gangrenous conditions, with much development of gas. See Chapter XIII.

γ. The bacillus of malignant œdema occurs in septic wounds with septicæmic complications. See Chapter XIII.

δ. The tetanus bacillus is found in the wound in cases of traumatic tetanus. See Chapter XIII.

ε. The typhoid bacillus is rare ; it may occur in suppurative conditions complicating or following typhoid fever. Proceed as in 8 and 4. (See also p. 346.)

ζ. When the *Bacillus pyocyaneus* is present the pus or discharge may be blue. Proceed as in 3 and 4.

(g) If yellow granules, having a rosette-like structure microscopically, are present, actinomycosis may be suspected and more particularly examined for by the methods given in Chapter XV.

(h) If thread forms be present, *streptothrix* or *aspergillar* infection may be suspected (see Chapters XV. and XVII.); if large round or ovoid cells or yeast-like forms, *Blastomycetes* (Chapter XVI.).

(i) If a mixture of organisms be present, agar and gelatin plate cultivations should be prepared and further examined by subcultures from the colonies.

(j) If no organisms can be detected microscopically, proceed as in 8, 7, or 9. In the pus of ordinary abscesses micro-organisms can generally be detected, unless caused by the tubercle or glanders bacillus, the pneumococcus, or by the *Amœba coli*.

3. Make several cultivations on agar and gelatin (anaerobic if required), and examine microscopically and by subcultures when the growths have developed.

4. Make two or three sets of agar and of gelatin plate cultivations. Examine the colonies microscopically and by subcultures.

5. Stain two or three of the cover-glass preparations by Gram's method, and counter-stain with Bismarck brown.

6. The gonococcus and *Diplococcus intracellularis* may be identified and distinguished by the methods detailed at pages 219 and 223.

7. Inoculate guinea-pigs or mice subcutaneously and intraperitoneally with the material.

8. Organisms can rarely be detected in the blood by a microscopical examination of stained films. Therefore 2-5 c.c. of blood should be withdrawn and cultivated (p. 117).

9. If the abscess be probably a tropical abscess of the liver, the pus or scrapings from the wall of the abscess should be examined for the presence of the *Amœba coli*. (Chapter XVIII.)

MICROCOCCUS MENINGITIDIS.¹

Weichselbaum in 1887 isolated from cases of epidemic cerebro-spinal meningitis (spotted fever) a coccus which he named the *Diplococcus intracellularis meningitidis*, and further research has confirmed the accuracy of Weichselbaum's discovery and the ætiological relationship of the organism to the disease.

Morphology &c.—The meningococcus, as it may be termed, occurs as single cocci and diplococci in groups within the leucocytes (Plate III., a); in grouping and general

¹ See Gordon, *Rep. Loc. Gov. Board*, 1907 (Bibliog.); Arkwright, *Journ. of Hygiene*, vii., 1907, p. 193.

appearance, in fact, it closely resembles the gonococcus, and, like the last named, is Gram-negative, though staining well with the ordinary anilin dyes and with the Leishman stain. In cultures it occurs as cocci, diplococci, and occasionally as tetrads.

Cultural Characters.—The meningococcus is an obligatory aërobe, and does not grow at a temperature below 25° C. It will occasionally grow in primary culture on glycerin agar, but frequently not, though when acclimatized it grows fairly well on agar and in broth. The organism develops best on agar smeared with blood, on ascitic fluid agar or broth, or on the nutrose ascitic agar of Wassermann (termed by Gordon 'nasgar'):

Ascitic fluid	15 c.c.
Distilled water	85 c.c.
Nutrose	1 gram

The mixture is placed in a flask, brought to the boil with constant shaking, and filtered. It is then mixed with double the volume of ordinary nutrient agar, steamed for thirty minutes, filtered, and filled into tubes.

The colonies of the meningococcus on this medium after twenty-four hours' incubation at 37° C. appear as moist, grey, translucent, circular or oval discs with regular outline; after a further twenty hours' growth they may attain a diameter of 3 to 4 mm. The colonies never exhibit any yellowish coloration, as do those of some other Gram-negative cocci. Ascitic fluid broth (ascitic fluid 1 part, broth 9 parts) is also a good culture medium, and it grows in milk without clotting or change in reaction. Arkwright found that grown in gelatin at 37° C. the meningococcus causes liquefaction, while the *M. catarrhalis* does not. The organism needs constant transplantation to maintain vitality in culture. The fermentation reactions are given in the table on p. 225.

Pathogenesis.—In man the organism causes epidemic cerebro-spinal meningitis, and is occasionally met with in sporadic cases of cerebro-spinal meningitis. It is also capable of producing a hæmorrhagic septicæmia without meningitis. It occurs in the cerebro-spinal fluid (obtained by lumbar puncture), in the blood in 25 per cent. of the cases provided quantities of 5 to 20 c.c. be cultured, sometimes in the upper respiratory passages, particularly the nose, in the middle ear, eye and joints. Park states that the organism is usually present in the nose in the early days of the illness. The meningococcus is pathogenic to mice and guinea-pigs by intraperitoneal or intrapleural, but not by subcutaneous, injection. Intraspinal injection into monkeys produces a typical meningitis.

An agglutination reaction is given in some cases, but is neither constant nor marked enough to form a sure means of diagnosis.

Symmers and Wilson¹ have found that the blood of epidemic cerebro-spinal meningitis cases may occasionally agglutinate the *B. typhosus* and *B. coli* in comparatively high dilutions.

Vaccine and Anti-Serum.—Cases have been reported of remarkable benefit derived by vaccinating with killed cultures.

Flexner has prepared an anti-serum with which successful results have been obtained.

Still observed in simple posterior basic meningitis of infants a diplococcus closely resembling the meningococcus but growing more freely on agar, &c. By some it is regarded as an attenuated form of the latter. According to Arkwright, it does not liquefy gelatin and grows on this medium at 22° C., fails to produce acid from glucose, maltose, and galactose, and is not agglutinated by a meningococcus serum. It is in these respects very like the *M. cinereus* of Lingelsheim. *Diplococcus crassus* (Gram-

¹ *Journ. of Hygiene*, viii., 1908, p. 314.

positive), *D. mucosus* (grows on gelatin), *D. flavus* (produces yellow pigment), and *M. catarrhalis*, the three latter Gram-negative, may occur in the naso-pharynx. (See Arkwright, *loc. cit.*, also p. 224.)

MICROCOCCUS GONORRHOÆÆ.

The *Micrococcus gonorrhœæ* was discovered by Neisser in 1879 in cases of gonorrhœal urethritis. In gonorrhœal pus it occurs usually in pairs, occasionally in tetrads, the elements of which are somewhat ovoid in shape, their opposed surfaces being flattened. The organism has a characteristic arrangement: it occurs in groups *within* the pus cells (Plate III., *b*). The individual cocci vary somewhat in size, the average being about $0.7\ \mu$ in the long and $0.5\ \mu$ in the short diameter. It stains readily with the ordinary anilin dyes, Löffler's blue being perhaps the best, but is decolorized by Gram's method—an important practical distinction from many other cocci.

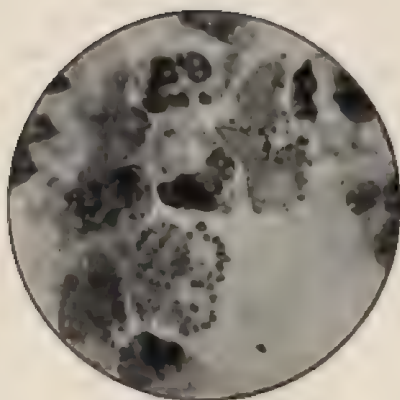
Cultural Characters.—The gonococcus is difficult to cultivate, and usually soon dies out under cultivation—within a week, unless transferred to fresh soil—but it does not seem to lose its virulence. Growth takes place between 25° and 38° C., but the optimum temperature is between 35° and 37° C. It is aerobic, and possibly facultatively anaerobic, and will develop on a feebly alkaline or acid soil. The ordinary agar and gelatin media are useless for the cultivation of the gonococcus; it will grow only on a medium containing 'native' protein. Blood-serum agar gives fair results, but the ordinary Löffler's blood serum is of no use. The best medium is agar smeared with blood. Ordinary sloping agar tubes or small agar plates may be employed. Blood obtained by pricking the finger, with antiseptic precautions, is taken up in a sterile capillary tube, and

deposited on the agar. A trace of gonorrhœal pus, collected with aseptic precautions, is taken up on a small sterile camel's-hair brush, and rubbed up with the drop of blood and smeared over the surface of the agar. The cultures are incubated at 37° C., and in twenty-four hours the colonies of the gonococci appear as transparent greyish specks, which increase in size up to the end of three days. At this stage the colony measures 1 to 2 mm. in diameter, is raised, brownish and finely granular in appearance, and roundish with a crinkled margin. The cocci from cultures resemble those in the pus, but tetrads are more frequently met with. The fermentation reactions and comparison with other Gram-negative cocci will be found in the table, p. 225. The specific virulence of gonorrhœal pus is destroyed by exposure to a temperature of 60° C. for ten minutes.

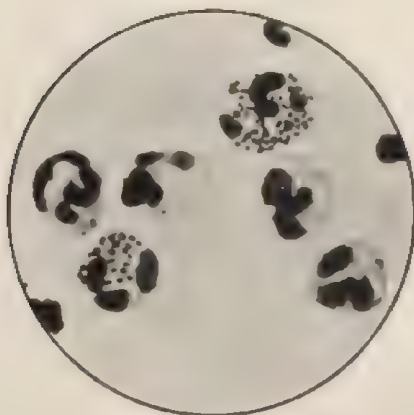
Pathogenicity.—The gonococcus is a strictly parasitic organism, and seems exclusively to attack man. From inoculation experiments on the human subject it appears to be the specific organism of gonorrhœal urethritis and vulvitis. In the female it is most frequent in the urethral or vulvar discharge, less so in that from the cervical canal, and rarely or never seen in a purely vaginal one. It is generally, even at an early stage, associated with other organisms, particularly other diplococci (see table, p. 225) which have to be distinguished from the gonococcus. The features which serve to identify the latter are its shape and size, its non-staining by Gram's method, its arrangement in *groups within* the pus cells, absence of growth on ordinary media, the characters of the colonies, and the fermentation reactions.

The gonococcus is associated with a variety of lesions besides those already mentioned, viz. epididymitis, ovaritis, salpingitis, cystitis, peritonitis, arthritis, and conjunctivitis. It has been met with in the blood, and occasionally produces endocarditis, pericarditis, and meningitis. The gonococcus

PLATE III.



a. THE MENINGOCOCCI.
SMEAR OF CEREBRO-SPINAL FLUID. $\times 1500$.



b. THE GONOCOCCI.
SMEAR OF GONORRHEAL PUS. $\times 1500$.



is fatal to guinea-pigs and mice by intraperitoneal inoculation.

Toxins, Anti-Serum, and Vaccine.—Christmas¹ found that the blood serum of the rabbit, fluid or coagulated, is an excellent culture medium for the gonococcus. By cultivating the gonococcus for ten days in an ascitic bouillon mixture he succeeded in obtaining a toxin which, when injected intravenously into rabbits in large doses, caused death, in smaller doses fever and loss of weight, while precipitated with alcohol and injected into the anterior chamber of the eye it produced severe inflammation. By injecting rabbits with small doses of the toxin, immunisation was produced, and the blood acquired antitoxic properties. A vaccine may be prepared by sterilizing cultures with heat, and has proved of service in chronic gonorrhœal infections.

CLINICAL DIAGNOSIS.

The diagnosis of gonorrhœa is very important, not only in clinical but also in medico-legal cases. For this purpose microscopical examination and culture methods are made use of. In a chronic gleet, the material must be examined carefully and repeatedly.

1. *Microscopical Examination.*—Several thin smear specimens of the pus or discharge should be prepared. If the best results are desired, the films should be air-dried, and then fixed by placing in a mixture of equal parts of alcohol and ether for fifteen minutes. After fixing, a couple of the films are stained in Löffler's blue for five to ten minutes, washed in water, dried and mounted. Leishman's stain also gives good results, the films being merely air-dried and not fixed. The preparations are then examined with a $\frac{1}{2}$ -inch oil-immersion; a lower power lens is useless. The ovoid cocci in pairs, and occasionally in tetrads, occurring within the pus cells in groups of not less than four pairs, are very characteristic. Diplococci situated outside the pus cells should be neglected (it

¹ *Ann. de l'Inst. Pasteur*, xi., 1897, p. 609.

is to be noted that the nuclei of the pus cells are deeply, the cytoplasm only faintly, stained with methylene blue). The next step is to ascertain the staining reaction by Gram's method. Stain two more films for fifteen minutes in anilin gentian violet, dip in water, place in Gram's iodine solution for two minutes, decolorize in absolute alcohol until the drainings fail to stain white filter-paper, and counter-stain for forty-five seconds in a saturated aqueous solution of Bismarck brown diluted with three times its volume of distilled water. The gonococci are decolorized, and take up the brown stain. In chronic urethritis the urine may be voided, and centrifugalized, and preparations made from the deposit and threads and stained.

2. *Culture Methods*.—Whenever a diagnosis is of great importance, an attempt should be made to cultivate the organism. Plate cultures of agar smeared with blood as described above, and another set with agar only should be prepared, and incubated at 37°C. In forty-eight hours colonies of the gonococcus should be recognizable on the blood agar, but not on the plain agar.

If cultures are obtained, the fermentation tests (p. 225) may be applied.

N.B.—*The greatest caution must be exercised in declaring a case free from infection on the ground of NEGATIVE results of the examination.*

MICROCOCCUS CATARRHALIS.¹

This organism occurs in the nose and throat in cases of catarrh, and particularly in the 'influenza cold' (see 'Influenza'), in bronchial catarrh, and occasionally in other conditions and in well people. Morphologically it occurs in pairs and tetrads, often within the polymorphonuclear leucocytes. It is Gram-negative. The primary generation develops feebly on agar, but subsequent generations grow fairly well, forming whitish translucent colonies. Blood or ascitic media should be used for isolation. Some of the fermentation reactions and a comparison with other Gram-negative cocci are given in the table, on the next page.

¹ See Gordon, *Brit. Med. Journ.* 1905, ii. p. 423; Arkwright, *Journ. of Hygiene*, vii., 1907. p. 145.

GRAM-NEGATIVE COCCI

225

The Characters of the Chief Gram-Negative Cocci (Gordon).

Organism or Source	Growth on Nutrose Ascitic Agar at 37° C.	Growth on Gelatin at 20° C.	Pathogenicity	Action on Carbohydrates + = acid - = alkali 0 = no action			
				Glucose	Galactose	Maltose	Saccharose
<i>M. catarrhali</i> . Nasal and pharyngeal dis- charge.	Opaque, granular.	Positive (grows on ordinary agar at 37° C.).	Mice and guinea-pigs by intra- peritoneal inoculation only.	-	-	-	-
<i>M. intracellularis</i> (Meningococcus). Cerebro-spinal men- ingitis.	Clear, smooth.	Negative	In some cases mice and guinea-pigs by intra- peritoneal inoculation only.	+	+	+	-
<i>M. pneumoniae</i> (Gono- coccus). Urethral discharge.	No growth unless blood added.	Negative	<i>Ib.</i>	+	+	0	0
From nasal discharge from Hertford case of influenza-like epi- demic (see 'Influenza').	Clear, smooth, later be- comes yel- lowish.	Negative at first, positive later (grows on ordinary agar at 37° C.).	Mice and guinea-pigs by intra- peritoneal inoculation.	+	-	+	-
<i>Ib.</i>	Opaque, granular.	Negative	<i>Ib.</i>	+	+	+	+
From urethra	Opaque, some- what granu- lar, smooth edges.	Positive	...	+	+	+	+
<i>M. melitensis</i> . Malta fever.	Creamy and slightly yel- lowish.	Positive	Monkeys. Also rabbits and guinea-pigs by intra- cerebral in- oculation.	-	0	0	0

MICROCOCOCCUS TETRAGENUS.

This organism is frequently met with in phthisical cavities and may be expectorated in the sputum, and has also been found in the pus of acute abscesses. The cells occur singly (diameter 1 μ), in pairs, or in fours, and enclosed within a capsule. It stains with the ordinary anilin dyes and also by Gram's method. On gelatin it develops slowly, with the forma-

tion of a thick, white, shining growth without liquefaction. On agar the growth has much the same characters, and on potato is white and viscous. Inoculated into animals, particularly mice, a local abscess may form, but usually it produces a fatal general infection, and the organism is found in the blood and organs.

A few cases of general infection in man have been described.

SARCINA VENTRICULI.

An organism occurring in the contents of the stomach, especially in cases of dilated stomach. Originally described by Goodsir in 1842.

It occurs as a large ovoid cell, several of which are grouped together quadrilaterally so as to form more or less cubical masses, the so-called 'woolpacks.' According to Falkenheim, it forms on gelatin in thirty-six to forty-eight hours roundish, prominent colonies of a yellowish colour. In neutral hay infusion it forms a brownish film and flocculi. It produces an acid reaction.

Other sarcine also occur in the stomach.

CLINICAL EXAMINATION.

1. The organism can be detected in the vomit &c. most readily by examination in the fresh state, a little of the material being placed on a slide, diluted with water if necessary, irrigated or not with iodine solution, covered with a cover-glass, and examined.

2. Cover-glass preparations may be stained with weak carbol fuchsin, or by Gram's method.

OTHER ORGANISMS MET WITH IN SUPPURATIVE AND SEPTIC CONDITIONS.

Many other organisms have been met with in various suppurative and septic processes, e.g. :

a. The *B. coli* in cystitis and pyelitis, ischio-rectal abscess, peritonitis associated with perforation and intestinal obstruction, and puerperal fever (see Chapter X.)

b. The *Diplococcus pneumoniae* in abscesses, empyema, arthritis, meningitis, pericarditis, peritonitis, &c. (see Chapter XII.)

c. The *B. typhosus* in abcesses, cholecystitis, empyema, and osteomyelitis (see Chapter X.)

d. The *B. oedematis* and *B. Welchii* in foul, gangrenous wounds (see Chapter XIII.)

e. The *B. tuberculosis* and *B. mallei* (see Chapter IX.)

f. The *Actinomyces* and *Streptothrix* forms (see Chap. XV.)

g. *Blastomyces* (see Chapter XVI.) and *Hyphomyces* (see Chapter XVII.)

h. The *Amoeba coli* (see Chapter XVIII.)

i. Capsulated bacilli (see note, p. 235).

COLEY'S FLUID.

This preparation consists of the toxins of the streptococcus of erysipelas and the *Bacillus prodigiosus*. It was devised by W. B. Coley, of New York, as a possible cure for inoperable malignant tumours. The treatment is based on the undoubted fact that malignant growths may decrease or even disappear completely after an attack of erysipelas (p. 210). The fluid is prepared by growing the streptococcus, obtained from a fatal case of erysipelas and rendered highly virulent by a succession of passages through rabbits, in bouillon for about ten days; the *prodigiosus* is then added, and the two are allowed to grow together for another week or ten days. The culture is finally heated to from 58° to 60° C. for one hour and a piece of thymol added to preserve it.

The fluid is injected subcutaneously in the vicinity of the tumour. The earlier injections may be performed with the *filtered* toxin, which does not produce so much reaction as the *unfiltered*.

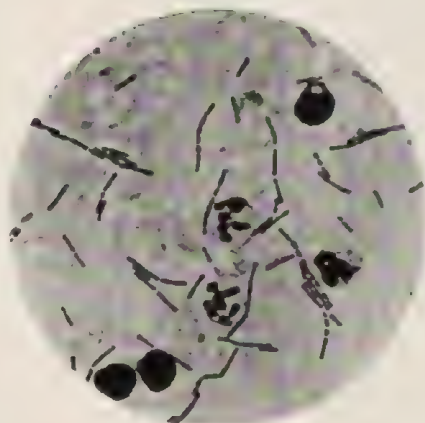
The dose to commence with should be 1 to 2 minims of the filtered, or $\frac{1}{2}$ minim of the unfiltered, fluid. The dose is gradually increased each day until there is a temperature reaction of 108° to 104° F. The temperature is the chief guide in estimating the dose, and the frequency of injections depends upon the general condition of the patient and upon the rapidity of recovery from the depression of the preceding dose. The injection must not be repeated until the temperature has completely fallen.

CHAPTER VII.

ANTHRAX.

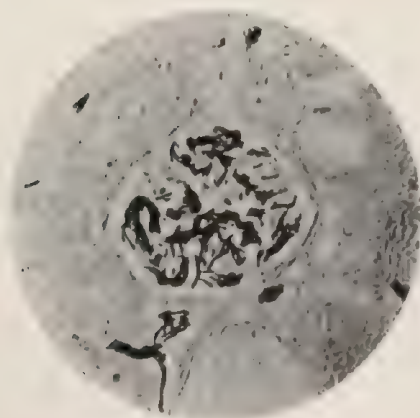
The disease known as anthrax claims attention not only on account of its importance, but also from historical considerations. It was perhaps the first disease to be definitely associated with a specific micro-parasite, for the anthrax bacillus was observed in the blood of infected animals as long ago as 1849 by Pollender, and in 1850 by Davaine, and the latter also claimed in 1863 to have demonstrated by inoculation experiments the causal relation of the bacillus to this disease. Davaine's experiments, however, cannot be said to have been conclusive, inasmuch as he had not complied with the second and third of Koch's postulates, which declare that the micro-organism must be cultivated outside the body, and the cultivated organism must produce the disease on inoculation. The experiments of Davaine were made by inoculating an animal directly with the blood from an infected animal, and the objection was therefore raised that infection was due not to the bacillus, but to something else in the blood. This objection was subsequently removed by the work of Pasteur and of Koch, who obtained pure cultures of the organism, the *Bacillus anthracis*, and with these produced results the same as had previously been obtained by inoculation with the blood of an infected animal. The disease in cattle is known as splenic fever, and though in England occurring only sporadically, or in small outbreaks, in some parts of

PLATE IV.



a. BACILLUS ANTHRACIS.

SMEAR OF BLOOD OF INOCULATED GUINEA-PIG. $\times 750$.



b. ANTHRAX.

SECTION OF KIDNEY THROUGH GLOMERULUS. $\times 500$.



the world its ravages assume serious proportions; as in Siberia, where it has been termed the Siberian plague, and at one time in France among the sheep to such an extent as to threaten the sheep industry with extinction. Man also suffers to some extent, and the disease is met with in persons whose occupation brings them into contact with animals, particularly cattle, or with animal products such as wool and hides.

Morphology.—The *Bacillus anthracis* is a rod-shaped organism varying slightly in size in different animals and under cultivation; in the blood it measures from 5 to 20 μ in length and 1 to 1.25 μ in breadth (Plate IV., a), but in cultures long filaments develop. Examined in the fresh and living condition in a hanging-drop preparation, these rods and filaments appear homogeneous or slightly granular; in stained preparations, however, they are seen to be made up of a series of segments with unstained interspaces, each segment measuring about 4 to 5 μ in length, and the ends of the segments appear cut off square, provided care has been taken not to overheat in fixing and to stain with an aqueous solution. In the blood the filaments never exceed about five or six segments in length, except perhaps in swine, in which animals they may be somewhat longer. In cultivations, however, the filaments may be of almost unlimited length and lie parallel to one other or in more or less tangled masses. In the animal body during life, and for some hours after death, spores never occur; but in cultivations more than a day or so old, and from which oxygen has not been excluded, they are always present, almost every segment containing one. The spores are ellipsoidal, measuring about 1 μ by 1.25 μ , and are centrally placed in each segment, the long axis corresponding with the long axis of the segment.

Cultural Reactions.—The anthrax bacillus is aerobic and facultatively anaerobic; it is non-motile, and stains

well with the ordinary anilin dyes, and especially so by Gram's method. It grows readily on all culture media at from 20° to 37° C., the latter being the optimum. Development ceases at temperatures below about 15° and above 45° C. Small, cream-coloured, granular colonies develop in a gelatin plate in about thirty hours, and in two

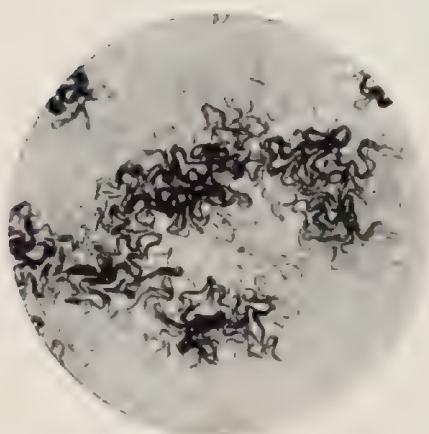


FIG. 35. — ANTHRAX. GELATIN STAB-CULTURE. SEVEN DAYS OLD.

to three days appear as small, roundish, cream-coloured pasty masses in little pits in the gelatin, due to its liquefaction. Microscopically, the colonies are somewhat characteristic; each consists of a mass of wavy, tangled filaments like a tiny wad of cotton-wool. In gelatin streak-cultures development is slow, and in four or five days a creamy, pasty growth forms in an area of liquefaction. In a gelatin stab-culture (preferably 5 per cent. gelatin) lateral branches spread from the central growth, longer in the upper layers, shorter below, so that at the end of a week the culture is like an inverted fir-tree (fig. 35) and the gelatin becomes gradually liquefied from above down-

wards. The colonies on an agar plate develop in twenty hours at 37° C. as cream-coloured points, which microscopically consist of little masses of wavy, tangled filaments (Plate V., *a* and *b*). On an agar surface culture at 37° C. there is a copious development in eighteen hours of a thick, cream-coloured, slimy growth, which at this early stage has a

PLATE V.



a. *BACILLUS ANTHRACIS.*
IMPRESSION PREPARATION OF A COLONY. $\times 40$.



b. *BACILLUS ANTHRACIS.*
IMPRESSION PREPARATION OF A COLONY. $\times 750$.



finely granular, ground-glass appearance. On blood serum a thick creamy layer forms, with slow liquefaction of the medium. On potato the organism grows freely as a dry greyish layer, with an abundant formation of spores. In broth it forms a somewhat scanty flocculent deposit, the broth remaining clear and giving the indole reaction.

In old cultures various involution forms are met with ; the rods lose their regular shape and become swollen, producing the so-called torula forms, while the homogeneous appearance of the protoplasm changes and becomes granular. Spores are found in all culture media when there has been free access of oxygen, as in surface cultures on potato and agar ; but in a deep broth culture, where the supply is limited, spore formation is absent or very scanty. During life spores are never met with ; they only appear some hours after death, or when the fluids containing the bacilli come in contact with air, as in the bloody discharge from the nostrils. It has therefore been supposed that oxygen is necessary for spore formation to take place, but this does not seem to be the whole explanation, for spores form in an atmosphere of nitrogen, though they do not do so in one of hydrogen. The life-history of the organism and the development of spores can be well watched in a hanging-drop specimen prepared by inoculating a droplet of broth with the blood of an infected animal. The preparation can be observed on a warm stage, or examined at stated times, being kept in the intervals in the blood-heat incubator. It will be found at the end of twenty-four hours that the short filaments, which alone are present in the blood, have grown so long that they stretch across the field, while the protoplasm has become granular, and minute shining points are visible here and there. In another twenty-four hours the filaments have extended, the protoplasm has become still more granular, and the shining spots are now well-marked ovoid, highly refractile bodies

the mature spores. In old cultures the rods and filaments almost disappear, numbers of spores alone remaining. These spores, when placed under favourable conditions of moisture, warmth, and nutriment, again produce rods and filaments; a little bud appears at the extremity of the long diameter, which grows in length and ultimately becomes a mature rod, with the empty spore capsule at one end. Sporeless varieties of the anthrax bacillus have been obtained by cultivating under unfavourable conditions, as at a high temperature (44° C.), or in the presence of minute quantities of antiseptics (1 : 1000 carbolic acid). The spores are of considerable practical importance, for they are highly resistant forms, requiring at least some minutes' boiling and three hours in dry air at 140° C. for their destruction, whereas the bacilli without spores are destroyed in ten minutes in the moist condition by a temperature of 54° C. The same resistance occurs towards various germicidal substances. While 1 per cent. carbolic acid and 1 : 10,000 corrosive sublimate solutions quickly destroy bacilli without spores, it requires at least 5 per cent. carbolic acid, acting for not less than twenty-four hours, and 1 : 500 sublimate solutions, acting for not less than an hour, both at 20° C., to kill the spores. Anthrax spores will retain their vitality and pathogenic power unimpaired for years in a dried condition.

Pathogenicity.—The anthrax bacillus is pathogenic for man, cattle, sheep, goats, rabbits, guinea-pigs, and mice. The horse and the pig are also susceptible; but adult white rats, dogs, cats, and Algerian sheep are immune. Young white rats, or rats fatigued by muscular work, can be infected, and frogs and fish, though immune under ordinary conditions, can be rendered susceptible by raising the temperature of their environment. Birds, such as fowls and pigeons, are also almost insusceptible, but may be rendered susceptible by lowering their temperature; smaller

birds, such as sparrows, are more susceptible. The virulence varies considerably and may be artificially modified in many ways; by passing through a series of susceptible animals it is heightened, by growing in the body of an insusceptible animal it is lowered, and the latter result is also obtained by cultivating for two or three weeks, at a temperature of 42° to 45° C., or by the addition of certain chemical substances to the culture medium—for example, 0.01 per cent. of potassium bichromate. These methods of ‘attenuation,’ as it is termed, are put to a practical use in the preparation of the anthrax vaccine.

Symptoms of the disease in cattle are not very marked. A beast may appear a little out of sorts and the next day be found dead, or after suffering for a day or two with general malaise, fever, and rigors, and with a sanguineous discharge from the nostrils and bowel, it dies suddenly. Post mortem, the chief feature that attracts attention is enlargement of the spleen, which may be two or three times larger than normal, is highly congested, and very soft and friable. Microscopically, the bacillus is found in enormous numbers in the spleen, somewhat less numerously in the blood, and still less so in the liver, kidney, and other organs. Swine do not often suffer from this disease, unless fed with the offal of an infected animal, in which case the chief clinical sign is great enlargement about the throat; this is almost pathognomonic, while the chains of bacilli tend to be somewhat longer than in other animals.

Mice inoculated subcutaneously usually die in about twenty-four hours, and enlargement and congestion of the spleen are very noticeable. An infected guinea-pig dies in from thirty-six to forty-eight hours and usually shows no symptoms until the last, when it may suffer from rigors, with high temperature, convulsions, and staring coat. Post mortem, the muscular tissue is found to be pale and cedematous, the spleen is enlarged to two or three times its normal

size and is highly congested and very soft, and minute hæmorrhages may occur in the serous membranes. Microscopically, bacilli are found throughout the spleen, and are often so numerous that in a stained preparation there appear to be more bacilli than tissue. Large numbers are also present in the blood and lungs, fewer in the liver and kidney; in the latter organ they are almost confined to the glomeruli (Plate IV., *b*). Immediately after death, however, comparatively few bacilli may be met with in the blood, the heart, and great vessels.

The spread of the disease in nature seems to result from the ingestion of spores while the animals are feeding. Although the bacilli would be destroyed by the acid gastric juice, this is not the case with the spores, which are probably generally developed from the organisms present in the bloody discharges of a stricken animal, and are distributed by wind and flood, and in this way may infect large tracts of pasture. Pasteur suggested that earthworms might bring the spores to the surface in their casts from the buried carcases of infected animals, but some experiments by Koch negated this. The non-sporing bacilli rapidly degenerate and die in a buried carcase.

Man seems to be relatively insusceptible to anthrax. The disease is generally met with among butchers, veterinary surgeons, shepherds, &c., and among those who sort wool or hair or work with or carry hides, e.g. glove-makers, tanners, porters, &c. The disease occurs in two forms — the so-called 'malignant pustule,' a cutaneous infection, not unlike an angry carbuncle, occurring at the seat of inoculation, on exposed parts of the body, such as the back of the neck, the face, wrists, and hands; and 'wool-sorter's disease,' a general infection, severe and fortunately rare, through the lungs or stomach. Rag-sorters are likewise sometimes attacked by anthrax, but there is also a distinct 'rag-sorter's disease' which is stated to be due to a non-motile,

non-sporing, non-liquefying, capsulated bacillus, the *Proteus capsulatus hominis*¹ of Bordoni Uffreduzzi.

In November 1896 the Home Office appointed a committee upon anthrax to make inquiry into, and report on, the conditions of work as they affect the health of the operatives in the industries in which anthrax is alleged to occur, and to report what, if any, special rules should be made or special requirements enforced under the Factories and Workshops Act, 1895, for the protection of persons employed in those industries, and as a result all cases of anthrax contracted in connexion with various industries have now to be reported to the Home Office. The matter has also been exhaustively dealt with by Legge.² It is particularly Persian wool, Chinese hides, and Russian hair which are dangerous, while Argentine, Australian, and New Zealand wools are almost innocuous. The sorting and exclusion of wool derived from infected animals seem to be impracticable, and the efficient sterilization of the thousands of bales that are imported an impossibility. As regards hides and skins, Legge points out that it is doubtful if there is any way in which hides to be afterwards tanned can be effectively disinfected, and to be of real benefit it would have to be done before the material is opened in the warehouse; but to secure this would be impossible. As regards horsehair, Webb and Duncan³ have carried out a number of experiments on its disinfection, from which it would seem

¹ Capsulated bacilli have been met with in many septic processes. This group includes Friedländer's pneumo-bacillus, *P. capsulatus hominis*, *B. mucosus capsulatus* of Fricke, and the *B. coli immobilis*. They are met with in conditions associated with sepsis, pus production, bronchopneumonia, ulcerating stomatitis, &c. They are shortish, non-motile, non-sporing rods, usually Gram-negative, easily cultivated and not liquefying gelatin, and in the tissues surrounded with a capsule.

² Milroy Lectures on Industrial Anthrax, *Brit. Med. Journ.* 1905, i. pp. 529, 589, and 641.

³ *Annual Report of Chief Inspector of Factories*, 1900, p. 472, and 1902, p. 278.

that, leaving out of consideration white or grey hair, which is liable to change colour, no injurious effect is produced on hair by steam disinfection, provided the temperature does not exceed 218° F.; but this is a comparatively low temperature for efficient disinfection, and success can then be obtained only with minute care in the construction and regulation of the apparatus. Legge concludes that to secure certain destruction of all anthrax spores in horse-hair absolute reliance cannot be placed on either steam disinfection (within the limits in which it can be applied) or simple boiling. Adoption of one or the other is a very material safeguard, but risk must always be run by those who prepare the hair for disinfection. Disinfection is now being attempted by subjecting the material to the action of disinfectants such as 5 per cent. cyllin and kerol.

A number of cases of anthrax, resulting in many deaths, have been reported in various parts of the United States, from tanneries dealing with hides imported from China. Also a number of cattle have been infected as the result of drinking water from rivers and creeks receiving the waste liquors from these works.

Houston¹ detected the anthrax bacillus in a catch pit in a hide factory at Yeovil, and in sewage and effluents and in the mud of the Yeo. It has also been met with in linseed cake and oats.

Toxins.—From pure cultures of the *Bacillus anthracis* Hoffa obtained small quantities of a ptomine, which produced fall of temperature and hæmorrhages, and Hankin isolated a proteose which in large amounts was fatal, but in small amounts conferred immunity to subsequent inoculation with living bacilli. Brieger and Frankel obtained a tox-albumin from animals dead of anthrax. Marinier,² by growing the anthrax bacillus in a solution of peptone,

¹ *Second Rep. Commtee. on Sewage Disposal*, 1902, p. 31.

² *Ann. de l'Inst. Pasteur*, ix. 1895, p. 533.

glycerin, and salts, and subsequently precipitating with ammonium sulphate, obtained a toxin which he states is neither protein nor basic, and is contained within the bacterial cells.

Sidney Martin,¹ by growing the anthrax bacillus in alkali albumin for ten days, obtained from the culture albumoses and an alkaloidal substance. From the bodies of animals which had died of the disease, chiefly from the spleen and blood, he obtained similar substances, the amount of alkaloid being more than double that of albumose. The mixed products produced fever in animals followed by coma and death. The albumose was proved to be the fever, and the alkaloid the coma, producer; the latter also caused a spreading cedema at the seat of inoculation.

Anti-Serum.—An anti-serum for anthrax was prepared by Marchoux by immunizing sheep or rabbits by vaccination and then inoculating with progressively increasing doses of virulent anthrax cultures, the quantity being doubled every eighth day, until ultimately 200 c.c. to 300 c.c. of a very virulent culture is given. The treatment has to be prolonged (five months), and the most active serum is obtained between two and three weeks after the last injection. Selavo has also prepared an anti-serum by first immunizing asses with a vaccine and then inoculating with increasing doses of virulent cultures. This serum has been used successfully in several cases of anthrax in man.

Vaccine.—An attenuated virus has been extensively employed for the vaccination of cattle and sheep. Cultures are attenuated by growing at 42°–43° C. (Pasteur, Chamberland, and Roux). A weak vaccine is first injected, followed after 10–12 days by an injection of a stronger vaccine. The mortality as a result of the vaccination is small, and

¹ 'Chemical Pathology of Diphtheria,' &c., Gulstonian Lectures, *Brit Med. Journ.* 1892, i. p. 641.

the animals are subsequently protected for some months against the virulent disease.

CLINICAL EXAMINATION.

1. *In Veterinary Practice.*—If an animal is suspected to have died from splenic fever, no extensive post-mortem should be made, because of the distribution of blood &c. containing the bacilli and the risk of the development of spores, and their subsequent dissemination, with infection of pasture. The abdomen should be opened and the spleen examined. If this is found to be much enlarged, and so soft that it can hardly be handled without rupture, there is a high probability of splenic fever, which the history of sudden death, with or without symptoms coupled with a sanguineous discharge, increases. To confirm the diagnosis, some smear preparations should be made from the spleen and blood, and can be stained and examined on arriving home. If slides or cover-glasses are not available, the ear or a small piece of the spleen may be removed and taken home, where the specimens may be examined. When material is sent from a distance for examination, the ear should be forwarded.

The smears may be stained with Löffler's blue and by Gram's method with eosin. The characteristic appearances of these preparations are numbers of large bacilli forming chains of five or six segments, and if the post-mortem has been made within a few hours of death no spores are visible. If the material has been kept, methylene-blue staining gives the most characteristic picture. *In such cases, large saprophytic bacteria somewhat resembling anthrax are always present and must not be mistaken for that organism.* If a hanging-drop preparation can be made, a characteristic is the non-motility of the bacilli.

The stained preparations can be kept and produced in a court of law if necessary. Cultivations can also be made from the spleen, but the necessary culture media are not of course usually forthcoming. Finally, a guinea-pig may be inoculated subcutaneously in the abdomen with a particle of the spleen, and after death examined microscopically and by culture methods.

As regards the disposal of the carcase of an animal dead from anthrax, this should be burned if possible, but, failing this, it

may be buried in a deep pit, preferably with plenty of lime. All traces of blood and discharge must be carefully mopped up with a strong lime-wash or solution of chloride of lime, or other reliable disinfectant.

2. *In Man.*—In malignant pustule, smear specimens should be prepared from the fluid of the vesicles or with the scrapings from the incised pustule, or sections of the excised pustule may be made, and stained, some with Löffler's blue, others by Gram's method with eosin. The bacilli are not often met with in the blood, except shortly before death. At the same time cultivations on agar and gelatin should be prepared, and may yield positive results when the microscopical examination has been negative. In the later stages of the disease, the bacilli may be difficult to find, even in sections.

In all cases of doubt a guinea-pig or mouse should be inoculated subcutaneously with the material, and if the animal dies, the diagnosis of anthrax may be confirmed by the characteristic appearances, by a microscopical examination, and by cultivation. The animal experiment is by far the most certain method of diagnosis, a negative result being nearly as valuable as a positive one.

N.B.—It must be noted that both cultivation and inoculation experiments may fail to give positive results, if the material be old or putrid.

CHAPTER VIII.

DIPHTHERIA.¹

Diphtheria in England—The Diphtheria Bacillus—The Pseudo-Diphtheria Bacillus—Clinical Diagnosis—The Xerosis Bacillus—Diphtheritic Affections of Birds and Animals.

Diphtheria seems to have been known from the earliest ages, being recognized by the classical (medical) writers, and it was epidemic in England and on the Continent during the Middle Ages. Bretonneau experienced an outbreak at Tours, 1818-1821, and gave to the disease the name 'Diphtherite' (afterwards changed to 'Diphthérie') from the formation of membranes which is so marked a feature in it.

In England the diphtheria deaths have only been separately scheduled since 1855. Since 1881, until recently, there has been a steady increase in the prevalence of diphtheria, particularly in the large towns, but latterly the prevalence seems to be decreasing.

As regards croup, it is universally admitted that the vast majority of cases of membranous croup are cases of diphtheria. In consequence, there has been a decided change in nomenclature, as is well seen in the following table:

Mortality in England and Wales per 1,000,000 living.

Period	Diphtheria ¹ Croup		Diphtheria and Croup	Laryngitis	Sore-throat and Quinsy
1861-70	185	246	431	51	14
1871-80	121	168	289	48	10
1881-90	163	144	307	54	24
1891-95	254	70	324	48	21
1896-1900	272	34	306	42	—
1901-1905	204	16	220	37	—

¹ See *The Bacteriology of Diphtheria*, Cambridge University Press, 1908.

² See *Memoirs on Diphtheria*, New Sydenham Soc. 1859.

Various writers have shown that a waterlogged condition of the soil, and dampness generally, favour the occurrence of diphtheria, the disease diminishing with drainage.

Diphtheria is distinctly a disease of the young, especially at the ages from two to ten, and this holds good both for London and for England and Wales.

Considerable difference of opinion has existed in the past as to the nature of the disease known as diphtheria. At present all are in the main agreed that it is an infective disease, but observers still differ as to what group of clinical characters constitutes diphtheria. The typical cases in which there is a washleather-like membrane, leaving a bleeding patch on separation, comparatively low temperature, prostration, and albuminuria, are frequently departed from, and the most experienced physicians are sometimes deceived by cases which run the course of a mild tonsillitis and afterwards develop paralysis. It is safer, and probably more correct, to regard diphtheria as a disease showing marked variations in the clinical manifestations, gravity, and sequelæ, and not to depend on any one symptom or group of symptoms for its diagnosis. That diphtheria is an infective disease is amply proved by the history of epidemics, and by the recorded cases where the disease has been conveyed from one individual to another.

The bacteriological study of diphtheria was commenced as long ago as 1882 by two German investigators, Klebs and Löffler. Klebs especially investigated the pathological histology, and ascribed the disease to small rod-shaped organisms, which he observed were present in the membrane. It was reserved for Löffler to place this observation of Klebs on a firmer basis by the isolation and cultivation of the bacillus from the membrane, and by the production of certain phases of the disease by inoculation with the isolated organism. The cause of diphtheria is, therefore.

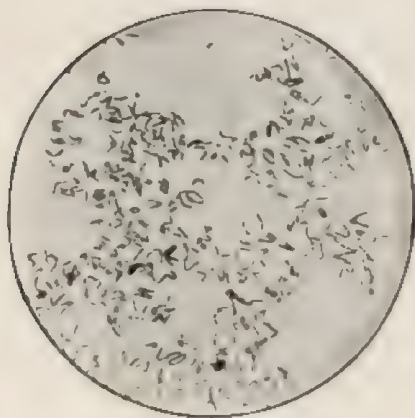
this diphtheria bacillus, which from its discoverers is frequently known as the Klebs-Löffler bacillus.

The isolation of the specific organism was by no means an easy matter, as a number of species of bacteria is frequently associated in the membrane. The organism can be readily isolated from the membrane by the employment of a special culture medium, Löffler's blood serum, which consists of a mixture of blood serum (ox serum was that originally used) 3 parts and glucose bouillon 1 part, the whole being coagulated (see p. 53). On this medium the diphtheria bacillus grows and multiplies exceedingly well, while the other organisms associated with it in the membrane are to a large extent inhibited in their growth. By rubbing a small piece of membrane from a case of diphtheria over the surface of two or three tubes, or of a plate of Löffler's serum, and incubating at 37° C. for twenty to twenty-four hours, colonies of the diphtheria bacillus will be found more or less isolated according to the number of organisms present in the membrane, and by subculturing from these, pure cultures may be obtained.

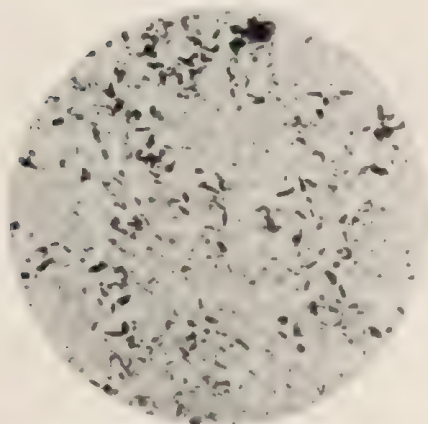
CHARACTERS OF THE DIPHTHERIA BACILLUS.

Morphology.—The *B. diphtheriæ* is a small, delicate bacillus, with rounded ends, measuring 3 or 4 μ in length. The size is somewhat variable even on the same medium, and three varieties of the bacillus have been described, viz. long, medium, and short, according to its length. Some of the rods both in cultures and in the membrane are usually somewhat swollen at one end, the so-called clubbing, and parallel grouping, both in the membrane and in cultures, is almost universal, the bacilli lying parallel side by side (Plate VI., a). This parallel arrangement arises from the peculiar mode of division of the bacillus. If a cell be observed upon a warm stage, it first

PLATE VI.



a. THE KLEBS-LÖFFLER OR DIPHTHERIA BACILLI.
COVER-GLASS PREPARATION OF A SERUM CULTURE. $\times 1500$.



b. SECTION OF DIPHTHERITIC MEMBRANE WITH KLEBS-LÖFFLER BACILLI.
GRAM AND EOSIN. $\times 750$.

elongates, then becomes constricted at about its middle, and then suddenly *one* side of the cell membrane seems to rupture and one half of the cell bends over to the other, so that the two halves form a V. This mode of division occurring in contiguous cells, and being repeated, and the cells thus becoming more and more crowded together, leads to the arrangement in parallel series. Distinct thread and branching forms, though of rare occurrence, may be met with, but it is very common for the bacilli to be joined end to end in a series of twos. On different media there are also considerable variations in size. On blood serum and on gelatin the bacilli are of medium length; in broth they tend to be short and stunted; while on agar, especially glycerin agar, they are much larger than on the former media. Variations in form are also almost invariably met with on culture media. This is especially marked on glycerin agar, long club-shaped and spindle-shaped rods being abundant; on blood serum they also occur, but sparsely in a young, eighteen to twenty hours' culture, in a forty-eight hours' culture more numerous. It is non-motile, does not form spores, and is aerobic and facultatively anaerobic.

Staining Reactions.—The *B. diphtherie* stains well with the ordinary anilin dyes and is Gram-positive. The most characteristic appearance, perhaps, is obtained by staining with Löffler's methylene blue. The coloration is usually somewhat irregular, more deeply stained portions alternating with paler intervals, the so-called segmentation, and especially marked with agar cultures. The ends of the organisms are also frequently more deeply stained than other parts, the so-called polar staining, while the phenomenon known as 'metachromatism' is often marked both at the poles and also in the rod, appearing as granules of a purplish tint and contrasting with the blue of the methylene blue. With Neisser's stain (p. 270) deep inky coloured dots,

appearing somewhat larger in diameter than the rods, occur at the poles of the organism and occasionally at the centre.

Cultural Reactions.—The diphtheria bacillus is an aerobic and also a facultatively anaerobic organism, and grows well on all the ordinary culture media, forming cream-coloured growths or colonies, the latter on serum tending to be somewhat flattened with regular margins. It grows slowly on gelatin, forming a raised whitish growth without liquefaction of the medium, and flourishes in milk, with the production of an acid reaction, but without curdling. In broth some strains give a granular growth on the sides and at the bottom of the tube, the broth remaining clear, sometimes with a thin surface pellicle; other strains may render the broth turbid throughout. On potato the growth is slight and invisible.

The indole reaction can be obtained in peptone water cultures either with or without a nitrite, but the writer has shown that this reaction is due, not to indole, but to skatole-carboxylic acid (see below, p. 264).

The diphtheria bacillus attacks glucose and lactose with the formation of acid, but no gas (see Table, p. 268). As regards the production of acid, Neisser found that during the first nine hours there is little or none; at the end of twenty-four hours a considerable quantity has been formed, and the amount increases until the end of the second day, after which the production ceases.

The *B. diphtheria* is agglutinated by the serum of patients and by a diphtheria serum, but the test is difficult to apply on account of the coherence of the growth, is somewhat erratic with different strains, and is of no practical value in the diagnosis of the disease, and little for the recognition of the organism and for distinguishing it from the so-called 'pseudo-diphtheria' bacilli.

The Klebs-Löffler bacillus retains its vitality in cultiva-

tion for a month, and when dried for three or four weeks. According to Welch and Abbott, it is destroyed in ten minutes by a temperature of 58° C. It is readily destroyed by antiseptics when in cultivation, but in the membrane it is difficult to find an agent which will penetrate and kill the bacilli beneath the surface.

The diphtheria bacillus and its characters under cultivation have been described somewhat fully, because of the importance of the identification of the organism as a means of clinical diagnosis. As mentioned at the commencement of this chapter, the clinical diagnosis of diphtheria presents many difficulties, and considerable assistance may be derived from a bacteriological examination. The diagnosis is based on the presence or absence of the Klebs-Löffler bacillus either in smears, or in cultivations, made from the membrane or secretion (see p. 268). This method is of very real assistance in doubtful, and especially in mild, cases, where clinically it would be very difficult to say whether the condition is diphtheritic or no. The mild cases are those which it is of the greatest importance to identify, especially in schools, for if not recognised the patients may go about and prove a source of infection to all around. The method also affords valuable evidence as to when a case can be considered free from infection; as long as bacilli are present in the throat, infection must be possible, and the length of time for which they may occasionally persist is remarkable. In half the cases the bacilli disappear within three days of the disappearance of the membrane, in a few cases they linger for as long as three weeks, but they may persist much longer. The writer isolated them for as long as five months (and virulent to the last); and a case is recorded in which they persisted for no less than fifteen months after the attack. In all cases, two or three examinations should be made at short intervals with negative results before the bacilli can be

pronounced to be absent, and no case should be discharged from hospital until the absence of bacilli has thus been proved. When bacilli persist, treatment with antiseptic sprays or gargles, especially an aqueous solution of 1-2000 corrosive sublimate, combined with syringing the nose, usually soon causes their disappearance. Syringing the nose is important, for the bacilli probably extend to the post-nasal space, where they are untouched by a throat spray or gargle. Another mode of treatment has also been adopted. A polyvalent *anti-microbic* agglutinating anti-diphtheria serum has been prepared, dried, and compressed into tablets, one of which is dissolved in the mouth every two hours, and fifteen minutes after solution the nasopharynx is flushed with physiological salt solution. This is stated to get rid of the bacilli.

With regard to the value to be attached to the bacteriological examination for diphtheria, while the finding of the bacilli is proof positive of the diphtheritic nature of the affection and its infective nature, their absence is not of so much value, as various circumstances modify the result. For example, an unskilled person may not happen to touch the right spot with the swab, or from struggling &c. on the part of the patient even a skilled operator may fail to reach any but a small portion of the mucous membrane, instead of obtaining a good mop from all over, especially when there are no definite patches of membrane. The use of antiseptic gargles or paints shortly before the swabbing is taken will likewise prevent the growth of the bacilli. It sometimes happens that a very mixed growth is obtained in the cultures, and in such cases the Klebs-Löffler bacillus may be missed. Bearing such sources of fallacy in mind, and making due allowances for them, the negative result of a bacteriological examination may have considerable value in those cases which clinically are doubtful. *In no case where there is a reasonable suspicion of diph-*

theria should treatment with antitoxin be delayed until the bacteriological report is obtained.

The bacilli from the throat are frequently associated with other organisms, especially micrococci and torulæ; and those cases in which the temperature tends to be high and the throat fetid are usually a mixed infection of diphtheria bacilli with the *Streptococcus pyogenes* or *Micrococcus pyogenes*, var. *aureus*. The fact of such mixed infection cannot, however, be definitely decided from the cultures, as these organisms may be present in the mouth or throat without necessarily taking part in the infective process. Nor can the severity of the disease be gauged from the characters or numbers of the diphtheria bacilli and other organisms present, though perhaps, in a number of cases, those which yield pure or almost pure cultures of bacilli will probably be more severe than the cases which yield cultures with few bacilli. It has been stated that the long form of the diphtheria bacillus is the most, and the short form the least, virulent, the medium being intermediate, but this is by no means a universal rule. Westbrook¹ has divided all forms of the diphtheria bacillus into three groups, distinguished by their staining reactions with methylene blue. Those with deeply staining granules he calls '*granular forms*,' those with transverse bands '*barred forms*,' and those staining evenly '*solid forms*.' Each group is further divided into seven types according to shape and size, the types being designated by the letters A to G and being progressively smaller from A to G.

It is sometimes stated that a microscopical examination, unless controlled by inoculation of the isolated bacteria, is unreliable. Such a statement is extremely misleading. If the bacilli which have been cultivated from a suspicious throat possess all the characters of diphtheria bacilli, inoculation experiments are not needed, and if they were

¹ *Rep. Minnesota State Board of Health, 1899-1900.*

performed with a negative result (i.e. the bacteria were not virulent) would prove nothing, for the bacilli from different parts of a culture from a throat often possess different degrees of virulence. Occasionally, it is true, even the expert may be in doubt about a particular bacillus, but such cases are rare and probably do not occur more than once in 100 cases. Here an inoculation experiment might help, but would be of no value if a negative result were obtained. It is absolutely essential in the microscopical examination for diphtheria to use a good lens, good illumination, and sufficient amplification, not less than 800-1000 diameters.

The result of the examination of specimens from 1000 cases of suspected diphtheria by the writer and Nolan¹ was, that in 587 cases the diphtheria bacillus was found, in 409 cases it was not found, and in four instances bacilli were observed the identity of which was doubtful. Thus 58·7 per cent. of the cases were true diphtheria. In 40·9 per cent., or about two-fifths, of the cases the diphtheria bacillus was not found, and the majority of these were probably not diphtheria. A few may have been diphtheritic, though for some reason or other the bacilli were not found, but the error so introduced would probably not amount to more than 2 or 3 per cent.

In 600 of the cases notes were kept as to the organisms present in the cultivations, and the results are set forth in the opposite table.

The results are only approximate, as they are based on the more or less brief examination necessary to determine the presence or absence of the diphtheria bacillus, and no special pains were taken to observe all the organisms which might have been present. It is noteworthy that the diphtheria bacillus was obtained practically in pure cultivation in no fewer than 216 out of the 353 cases in which it was found. In only six was it associated with the streptococcus

¹ *Brit. Med. Journ.*, 1896, i. Feb. 1.

alone; but too much stress must not be laid on this point, for in a number of instances the mode of transmission of the specimen was not favourable to the vitality of the streptococcus, and in others it was doubtless overlooked, or perhaps included among micrococci. All the forms of the diphtheria bacillus were met with.

The following Organisms were present alone or associated with the <i>B. diphtherie</i> :	Cases in which the Diphtheria Bacillus was present, alone or associated with other Organisms	Cases in which the Diphtheria Bacillus was absent
<i>Bacillus diphtherie</i> alone	216	Pseudo-Diphtheria 2
Streptococci	6	32
Micrococci	55	79
Bacilli	19	41
Torula	9	1
Sarcinae	6	2
Streptococci and micrococci	2	23
Micrococci and bacilli	9	19
Streptococci and bacilli	1	5
Torula and bacilli	1	3
Sarcinae and bacilli	0	3
Micrococci (including streptococci) and sarcinae	6	8
Micrococci (including streptococci) and torula	4	14
Many forms present together	19	15
	353	247

Pathogenicity.—The diphtheria bacillus is pathogenic for man, the horse, ox, rabbit, guinea-pig, cat, chicken, pigeon, and finches, which are all more or less susceptible, while mice and rats are immune. In man the respiratory tract is usually affected, though the conjunctiva and other mucous membranes, as in the vagina and stomach, and wounds may be attacked. A membrane usually forms, consisting of laminae of fibrin with a few leucocytes and other cells, and here and there small effusions of blood, with coagulative necrosis of the underlying mucous membrane, and in this membrane the bacilli are for the most part located in the superficial layers (Plate VI., *b*), though in

all cases in which the disease has lasted for any time they are found in the lungs, spleen, and kidneys, and may occur even in the blood. If the patient recovers from the diphtheritic attack, paralytic sequelæ are far from uncommon, and are due to a peripheral neuritis. Membranes may be formed by other organisms, especially the streptococcus, also by the pneumobacillus, and occur in Vincent's angina (p. 272), but it is doubtful whether paralytic sequelæ follow any but a diphtheritic infection.

Some remarkable skin affections of an eczematous or ichthyomatous nature have been found by Hare¹ and others to be due to the diphtheria bacillus.

Another condition which seems to be generally diphtheritic is membranous rhinitis. Whereas true nasal diphtheria is a most serious condition, membranous rhinitis is seldom, if ever, attended with any risk to life, sequelæ do not occur, and it is rare to obtain a history of infection from cases of it. This is extraordinary and very difficult to explain, for the nose and nasal secretion swarm with virulent diphtheria bacilli.

Diphtheroid organisms can occasionally be isolated from well people and those not known to have been in contact with diphtheria cases. The Klebs-Löffler bacillus can be isolated from the throats of nearly 7 per cent. of the presumably healthy population,² in the throats of contacts the percentage rises to 33 or more. Murray and the writer³ found diphtheria-like bacilli in 58 out of 385 children (15 per cent.) admitted into the Victoria Hospital, Chelsea.

Ford Robertson believes that diphtheroid organisms—possibly the Klebs-Löffler bacillus itself—may play an

¹ *Lancet*, 1908, i. p. 282.

² See Eyre, *Brit. Med. Journ.* 1905, ii. p. 1101.

³ *Brit. Med. Journ.* 1901, i. p. 1174. See also Graham-Smith, *Journ. of Hygiene*, iii. 1903, p. 216.

important part in the production of general paralysis of the insane. His views have not gained general acceptance, and Eyre (*loc. cit.*) found that the percentage incidence of all diphtheroid organisms, and of the true *B. diphtherie*, in the throats of the insane was not greater than in well persons, and was unable to isolate the latter post mortem from cases of general paralysis.

Guinea-pigs are the animals generally employed for experimental purposes, and in order to compare the effects of various bacilli it is customary to make the inoculation with a measured volume of a forty-eight hours' broth culture. From 0.1 c.c. to 2 c.c. of such a culture, according to the virulence, inoculated subcutaneously, is usually required to kill a 250-gram guinea-pig within forty-eight hours. At the seat of inoculation an extensive hæmorrhagic œdema forms, and hæmorrhages occur in the serous membranes, and especially in the adrenals, while the renal epithelium and the liver cells undergo cloudy degeneration.

Inoculated into the trachea of the guinea-pig, rabbit, and chicken, pseudo-membranes form, and the same occurs with the superficially injured conjunctiva and vagina. It is stated by some that the diphtheria bacillus does not develop on a normal mucous membrane—this must first be injured, and the staphylococcus and streptococcus, so often associated with the diphtheria bacillus in the human subject, may play a part in preparing the way for infection by damaging the cells and tissues. Rabbits usually live somewhat longer than the guinea-pig after inoculation, and paralysis frequently develops if life is prolonged, simulating the post-diphtheritic paralysis of man.

The question of the occurrence of the Klebs-Löffler bacillus in the lower animals is of considerable importance with regard to the spread of the disease and the conveyance of infection. The so-called diphtheritic affections of pigeons, poultry, and calves (referred to more in detail below, p. 274)

have certainly nothing to do with human diphtheria. A number of observers assert, however, that cats may suffer from the disease, which in these animals runs a chronic course, and is associated with bronchitis, lobular pneumonia, nephritis, and wasting. Klein¹ points out that not only are cats liable to the disease in houses where diphtheria has occurred, but that a similar infectious disease exists naturally among cats, and symptoms similar to this natural disease may be produced by inoculating healthy cats with the Klebs-Löffler bacillus. The diphtheria bacillus has also been isolated from the horse.²

Several epidemics of diphtheria seem to have been traced to an infected milk supply. In some instances the infection has undoubtedly been derived from contamination from a human source, but in others this mode of infection could not be demonstrated, and it has been suggested that certain eruptive conditions on the teats and udder of the cow may be caused by the Klebs-Löffler bacillus and the milk become infected therefrom. Klein³ has made some experiments with a view of determining this point. He inoculated healthy cows in the shoulder with a bouillon culture of the diphtheria bacillus. This caused fever and local swelling, and in about a week a papular and vesicular eruption appeared on the udders and teats. The *B. diphtheria* was isolated from the contents of the vesicles and also from the milk on the fifth day, but not subsequently. The cows died in two to four weeks, and the *B. diphtheria* was obtained from the local lesions. Abbott⁴ obtained somewhat different results, but Klein⁵ points out that these experiments were not performed under exactly the same conditions as his own.

¹ *Rep. Med. Officer Loc. Gov. Board for 1889*, p. 162.

² *Cobbett, Centr. J. Bakt.* xxvii. No. 10, p. 631.

³ *Rep. Med. Officer Loc. Gov. Board for 1889 and 1890*.

⁴ *Journ. Path. and Bact.* ii. 1894, p. 35. ⁵ *Ibid.* p. 428.

Klein, Eyre, Dean, and Marshall,¹ have isolated the diphtheria bacillus from milk. It is to be noted that diphtheria-like, but non-pathogenic, bacilli are often to be found in milk and cheese (see section on Milk).

Toxins. Diphtheria toxin has not been obtained in a state of purity and its exact chemical nature is unknown. Löffler first investigated the chemical products formed by the diphtheria bacillus, and by precipitating bouillon cultures with alcohol obtained a white toxic substance which he classed among the enzymes.

Roux and Yersin precipitated the toxin from filtered broth cultures by means of absolute alcohol, and also by the addition of calcium chloride. They found that 0.4 mgrm. was sufficient to kill eight guinea-pigs or two rabbits, and considered it to be an enzyme.

From the blood and spleen of cases of diphtheria Sidney Martin² isolated albumoses (chiefly deutero-albumose) and an organic acid, but no basic body. Injected subcutaneously the albumose produces much œdema and irregularity of temperature; in larger doses depression of temperature with paralysis and coma. Small multiple doses, not sufficient to destroy life, may give rise to some fever, and in two or three days to paralysis of the hind legs in rabbits, with general weakness and loss of weight. Post mortem, the nerves are found to have undergone degeneration—breaking up and disappearance of the myelin and interruption of the axis cylinder, while the heart is fatty. The organic acid is also a nerve poison, but is not so toxic as the albumose. From diphtheritic membrane, extracted with 10 per cent. salt solution, only traces of albumose and organic acid were obtained, but the extract was highly toxic, producing fever and paralysis. Sidney Martin suggests that a body of the nature of a ferment may be present, and that

¹ *Journ. of Hygiene*, vii. 1907, p. 32 (Refs.).

² *Brit. Med. Journ.* 1892, i. p. 641.

the ferment in the membrane on absorption may perhaps form the albumose in the body. From cultures of the diphtheria bacillus in alkali-albumin, albumose and organic acid, with similar actions to those isolated from the body, were obtained.

Brieger and Frankel (1890) were unable to find any basic substance in cultures, and concluded that the toxic substance was a protein body, which they designated a 'tox-albumin.' It was destroyed by a temperature of 60° C. but not by one of 50° C., even in the presence of an excess of hydrochloric acid, and hence is probably not an enzyme. The tox-albumin is non-dialysable, is precipitated by saturation with ammonium sulphate but not with magnesium sulphate, and hence is neither a peptone nor a globulin, contains a large amount of sulphur, and gives the biuret and Millon's tests. A curious property of this body is that small quantities (2.5 mgrm. per kilogram of the body weight) do not produce their effects until the lapse of weeks. Brieger and Boer in a later research prepared the diphtheria tox-albumin by precipitating a bouillon culture with a 1 per cent. solution of zinc sulphate or chloride. The precipitate of the zinc double salt was washed with slightly alkaline water and decomposed with a stream of carbonic acid gas. The purified tox-albumin gives the xanthoproteic, biuret, and Adamkiewicz's reactions, and the red coloration on heating with Millon's reagent.

Frankel in some researches on immunity came to the conclusion that cultures contain two protein bodies, one of which is poisonous and is destroyed at a temperature of 65° to 70° C., while the other gives immunity and is not destroyed at this temperature.

The toxin broth is probably a complex mixture of toxic constituents belonging to the proteins (see p. 257), its poisonous property is destroyed in five minutes by boiling,

at lower temperatures more slowly, by light, and on keeping gradually diminishes.

Diphtheria Antitoxin.—By the injection of sub-lethal and increasing doses of the toxin into an animal an antitoxin is generated. For the preparation of a potent antitoxin for therapeutic use the first essential is a highly toxic toxin; for this a diphtheria bacillus of high virulence is required, and but few strains possess the necessary virulence. The virulent bacillus is grown in an alkaline broth (rendered alkaline to the extent of about 8 c.c. of normal caustic soda solution per litre beyond the neutral point to litmus) for about a week at 37° C. Various small points have to be attended to in order to obtain toxin of maximum toxicity. The use of meat some days old has been advocated, or of acid beef-broth in which *B. coli* has been grown for twenty-four hours, in order to get rid of the glucose. L. Martin makes use of 'peptone' prepared by the auto-digestion of a pig's stomach with dilute hydrochloric acid. Free access of oxygen is essential, a condition which may be obtained by growing in Erlenmeyer flasks in a comparatively shallow layer of broth. The cultures are then filtered through a Berkefeld or Pasteur-Chamberland filter to remove the bacilli. The filtrate is germ-free and very toxic, and a little carbolic acid may be added to preserve it. In New York 10 per cent. of a 5 per cent. solution of carbolic acid is added to the culture, the bacilli are allowed to deposit by standing for forty-eight hours, and the culture is filtered through paper; in this way filtration through a filter-candle is dispensed with. Less than 0.01 c.c. of the toxin should kill a 250-gram guinea-pig. Selected horses which have been tested with mallein and tuberculin, and kept under observation for some time to see that they are quite healthy, are then inoculated with this filtrate, commencing with a dose of 0.5-1 c.c., or 20 c.c. of the toxin together with 10,000 units of antitoxin may be given for the first three

doses. Individual horses vary very much in their susceptibility to the toxin, so that care has to be exercised with the first injections. The injections are given subcutaneously over the shoulder, and produce a local swelling and some rise of temperature and general disturbance, lasting two or three days. When this has passed away the inoculation is repeated, a larger dose being administered provided the reaction due to the former one was not too severe. The treatment is continued for five to six months, the dose of toxin administered being gradually increased until it may attain 500 c.c. or more. Cartwright-Wood found that by growing virulent diphtheria bacilli for three or four weeks in ordinary peptone broth, with the addition of 10 or 20 per cent. of blood serum or plasma, subjecting the culture to a temperature of 65° C. for an hour and filtering before injection, much larger initial doses can be given and some degree of immunization attained, and subsequently the ordinary broth cultures may be injected in large doses. Individual horses vary much in their capacity to yield antitoxin: on the whole those that are moderately sensitive to the toxin seem to produce most antitoxin; a horse to be of value should, after three months' treatment, yield an antitoxic serum containing not less than 300 units per c.c. The required potency having been attained, as shown by the tests to be immediately described, the horse is bled with aseptic precautions, the blood allowed to coagulate, the serum drawn off and filled into sterile bottles each containing a dose of the antitoxic serum. A small amount of antiseptic, such as tri-kresol, is generally added as a precautionary measure to prevent the multiplication of any stray germs that may have gained access during the various manipulations.

The potency of diphtheria antitoxin is now described in 'units' and is estimated by ascertaining the quantity of antitoxin required just to neutralize a certain amount of

a standardized toxin when injected into a 250-gram guinea-pig. Formerly, by Roux's method, the minimal fatal dose of the toxin was first ascertained, and the number of grams of guinea-pig which 1 c.c. of antitoxin would protect against the minimal lethal dose found. If 0.01 c.c. of antitoxin protects a 300-gram guinea-pig against the minimal lethal dose, 1 c.c. would protect $300 \times 100 = 30,000$ grams of guinea-pig, and the immunizing value of the antitoxin would be described as 30,000. This method is open to the fallacy that if only a portion of the lethal dose were neutralized, the guinea-pig might survive, and a fictitious value be given for the potency of the antitoxin. Behring later adopted ten minimal lethal doses as the test dose of toxin, and one-tenth of the amount of antitoxin which protected a guinea-pig against the ten minimal lethal doses, he described as a unit (the Behring unit, which therefore = 100 minimal lethal doses of toxin), from which the Ehrlich unit, now universally adopted, was derived. Ehrlich, however, found that in diphtheria toxin there are substances other than toxin which combine with antitoxin, though non-toxic or comparatively so, and that these substances vary in amount in different toxin broths; variable results, therefore, may be obtained by the simple method of testing. These substances, having an affinity for antitoxin, are termed toxoids, and are of several varieties, viz. (1) those having a greater affinity for antitoxin than toxin itself, protoxoids; (2) those having the same affinity, syntoxoids; and (3) those having a less affinity, epitoxoids.¹ Toxoids are probably derivatives of toxin; they increase in quantity in old toxin broth which has been kept, and which at the same time decreases in toxicity. Ehrlich also

¹ On the constitution of diphtheria toxin &c., see Ehrlich, *Die Werthbestimmung des Diphtherieheilserums* (Abdruck, *Klin. Jahrbuch*, vi. 1897), and *Refs.* p. 151. See pp. 153-156 for other views on the constitution of diphtheria toxin.

distinguishes certain other bodies, toxones, in toxin broth which also combine with antitoxin, having a less affinity for it than toxin, are *primary* secretory products of the diphtheria bacillus, and, while not lethal, induce induration, necrosis and paralysis. Ehrlich, therefore, devised a method of standardization which eliminates irregularities due to the variable proportions of toxoids, toxones, and toxin in the toxin broth, and antitoxin and not toxin was adopted as the standard, because of its better keeping qualities. In order to standardize an antitoxin, a fairly virulent toxin broth is first prepared and its minimal lethal dose roughly ascertained—i.e. that amount which is just sufficient to kill a 250-gram guinea-pig on the fourth or fifth day. Then, a solution of accurately standardized antitoxin, which can be obtained from the Serumsprüfung Institut, Frankfort-on-Maine, is prepared containing one 'unit' of the antitoxin in 1 c.c., and the toxin is standardized with this by mixing with it various quantities above and below one hundred minimal lethal doses. It is required to ascertain the amount of the toxin broth which, when mixed with one unit of antitoxin, just suffices to kill a 250-gram guinea-pig on the fourth or fifth day after the injection of the mixture; this amount of toxin is known as the L_+ dose. The L_+ dose may be defined as that amount of a given diphtheria toxin which is not completely neutralized by one 'unit' of standard antitoxin to the extent that *exactly* one simple lethal dose of toxin remains unneutralized; it corresponds usually to 105-120 minimal lethal doses. For example, suppose 0.003 c.c. of the toxin was found to be the minimal fatal dose, with separate 'units' of standard antitoxin, 0.2, 0.3, 0.4, and 0.5 c.c. respectively of the toxin might be mixed, and each mixture injected into a guinea-pig; probably the guinea-pigs receiving the 'unit' of antitoxin *plus* 0.2 and 0.3 c.c. of toxin would remain alive, while the animal receiving the 0.4 c.c. of toxin would die in twenty-

four to forty-eight hours. The death in the last case is too rapid, more than a simple lethal dose has remained unneutralized; and therefore the L_+ dose of toxin lies between 0.3 and 0.4 c.c., and further experiments would have to be performed with amounts of toxin between these limits in order to ascertain the exact dose. Death of the guinea-pig on the fourth or fifth day has been chosen because it has been found that if the dose of toxin be diminished ever so little below that producing this result, death does not ensue under nine or ten days. That is to say, an *acute* intoxication is fatal at the latest on the fourth or fifth day, a fatal result after then being due to a *chronic* intoxication. The amount of toxin which is *exactly* neutralized by one 'unit' of the standard antitoxin is known as the L_0 dose. By exact neutralization is meant absence of any reaction, general, or local at the seat of inoculation, in the inoculated guinea-pig. If toxin broth were a single substance, containing only toxin, then $L_+ - L_0 = D$, the simple lethal dose, would be equal to the minimal lethal dose. But because toxoids and toxones, substances also having an affinity for antitoxin, are likewise present, D , the difference between the L_0 and the L_+ doses, is usually a multiple (8-12) of the minimal lethal dose.

From these considerations we are now in a position to define the unit of antitoxin: a 'unit' is that amount of antitoxin which would neutralize about 100 minimal lethal doses for the guinea-pig of diphtheria toxin. From certain considerations, Ehrlich considers that the unit would exactly neutralize 200 minimal lethal doses of a theoretical toxin, containing only toxin and neither toxoid nor toxone, but, inasmuch as such a toxin is unknown practically, the unit corresponds usually to 105-120 minimal lethal doses of a toxin broth, the extremes which Ehrlich has found being 16 and 136 lethal doses. Having standardized a specimen of toxin by means of standard antitoxin, this standardized

toxin is in its turn used to standardize the antitoxic serum which has been prepared for therapeutic use. The toxin is preserved by the addition of toluol, and is kept in a cool dark place; it needs to be re-standardized every few weeks.

In standardizing antitoxin, the L_+ dose of the standardized toxin is mixed with varying amounts of the antitoxin, the mixtures are injected into guinea-pigs, and the amount of the antitoxic serum which neutralizes the L_+ dose of toxin is thus ascertained. If, for example, it were found that 0.05, 0.04, and 0.03 c.c. of the antitoxic serum neutralized the L_+ dose of toxin, but that the guinea-pig receiving 0.025 c.c. suffered from some local necrosis, wasted, and died in a few days, and the animal receiving 0.02 c.c. died in two or three days, 0.03 c.c. of this antitoxin would be about equivalent to one unit of standard antitoxin, and the antitoxic serum therefore contains 33 units per c.c. For all the experiments the conditions must be kept as constant as possible, guinea-pigs weighing 250 grams or thereabouts employed, and to eliminate irregularities a number of animals must be used. The antitoxic constituent of diphtheria antitoxin is globulin in nature, or is intimately associated with the globulin content of the serum. Thus Atkinson found that if the serum is precipitated by saturation with magnesium sulphate, the whole of the antitoxin is carried down with the precipitate, and also that the globulin content of the blood serum of antitoxin horses is increased. His results have been confirmed by Ledingham.¹

There can now be no doubt as to the value of the antitoxin treatment of diphtheria. Since the introduction of antitoxin treatment, which was commenced about the middle of 1894, there has been a steady decline in the case mortality from diphtheria, especially in London, where probably the

¹ *Journ. of Hygiene*, vii., 1907, p. 65.

majority of the cases are injected with antitoxin, as the following table shows :

Period	Death rate per 1000 living	Case rate per 1000 living	Case mortality per cent.
1891	0.31	1.5	30.6
1892	0.44	2.0	29.3
1893	0.74	3.2	30.4
1894	0.61	2.6	29.3
1895	0.52	2.6	22.8
1896	0.59	3.1	21.2
1897	0.50	3.0	17.7
1898	0.39	2.7	15.4
1899	0.43	3.1	13.9
1900	0.34	2.7	12.3
1901	0.29	2.68	11.1
1902	0.25	—	11.0
1903	0.16	—	9.7
1904	0.16	—	10.0
1905	0.12	—	8.3
1906	0.15	—	8.8

Not less than 2000 units should be injected for a dose, and early treatment is of paramount importance. As soon as there is a reasonable probability that the case is one of diphtheria the antitoxin should be used, and treatment should not be delayed for the result of the bacteriological examination. The statistics show that in cases treated on the first day of the disease, the case mortality is nearly nil, on the second day it is 4.3, on the third day 11.12, on the fourth day 17.24, per cent.

In bad cases, and in those coming under treatment at a late stage of the disease, the dose may be increased to 10,000, 20,000, or even 30,000 units with advantage, and to bring the patient under the influence of the antitoxin as rapidly as possible the first dose may be administered intravenously. The dose may have to be repeated once or twice in mild cases, in bad cases perhaps every six or twelve hours until several doses have been given, the guide being the general condition of the patient and the rapidity of the separation of the membrane.

In addition to antitoxin, the recumbent posture and general and local treatment should be pursued as usual.

In cases of mixed infection, where the diphtheria bacilli are associated with streptococci or staphylococci, diphtheria antitoxin may prove of less value, as it has no influence on the streptococcic or staphylococcic infection, and injections of anti-streptococcic serum may be given in addition.

Diphtheritic paralysis seems to be rather more frequent after the use of antitoxin than in cases not treated with it, probably because a greater number of cases survive.

The antitoxin has also been employed as a prophylactic, in schools or other places where susceptible individuals are congregated together, and where cases of diphtheria have occurred, with excellent results. For this purpose a dose of about 500 units should be given. The immunity so produced does not last for more than about a fortnight.

The procedure in such circumstances should consist of a bacteriological examination of the throats of all, staff and inmates, in the institution, isolation of those in whom the *B. diphtherie* is found, and the injection of everyone with a prophylactic dose, repeated, if considered desirable, a fortnight later.¹

Some clinicians assert that antitoxin may be given by the mouth or the rectum. Hewlett was unable to detect any absorption of tetanus antitoxin from the stomach or rectum, nor Sternberg of diphtheria antitoxin from the rectum, of rabbits.

Escherich and others have found the blood after an attack of diphtheria to be slightly antitoxic. The blood of persons never known to have suffered from diphtheria is also occasionally antitoxic, in explanation of which it may be suggested that diphtheria bacilli may have been present for a time without producing diphtheria and have led to the production of antitoxin.

¹ On the prophylactic use of antitoxin see Norton, *Lancet*, 1907, ii. p. 85.

PSEUDO-DIPHTHERIA AND DIPHTHERIA-LIKE BACILLI.

Diphtheria-like bacilli are not uncommon in wounds and in pathological exudates &c., and in connexion with diphtheria an important question must be discussed, viz. the occurrence and nature of the so-called pseudo-diphtheria bacilli. The term was originally used by Löffler, and by the rule of priority should be reserved for the organism described by him under this name. The pseudo-diphtheria bacillus of all authors is a non-virulent organism occurring in the throat in various anginal conditions, scarlet fever &c., and occasionally in healthy throats. Park and Beebe met with it in twenty-seven out of 330 healthy throats examined by them. Roux and Yersin, Abbott and Fränkel, describe it as morphologically resembling the Klebs-Löffler bacillus, while Löffler, von Hofmann, Koplick, Park and Beebe, Peters, and Hewlett and Miss Knight,¹ consider that an organism differing somewhat from the Klebs-Löffler bacillus should alone be termed the pseudo-diphtheria bacillus; to avoid confusion it is best to designate it the Hofmann bacillus.

Morphology.—Typically the Hofmann bacillus is a shortish rod tapering towards the ends which are rounded, the average length being from $1.5\ \mu$ to $2\ \mu$, and it occurs in pairs, resembling two suppositories placed base to base. It is non-motile, does not form spores, is arranged in a parallel grouping like the Klebs-Löffler bacillus (due to the same mode of division), and involution forms are, as a rule, not met with (Plate VII., a). It is Gram-positive, and stains deeply and regularly with Löffler's methylene blue, segmentation and polar staining usually being absent. With Neisser's stain no inky granules are perceptible, as is the case with the diphtheria bacillus.

¹ *Trans. Brit. Inst. of Prev. Med.* i., 1897.

Cultural Reactions.—The Hofmann bacillus is almost a strict aërobie; there is no growth anaërobically in hydrogen. On serum, agar, and gelatin it forms cream-coloured colonies or growths, barely distinguishable from the Klebs-Löffler bacillus. On ordinary potato it hardly grows at all, what growth there is being quite invisible. On alkaline potato,¹ however, it forms distinct cream-coloured colonies, usually visible by the second day. In stab-cultures in gelatin and glucose-agar no gas is formed, and the growth is confined to the upper part of the stab. In broth it forms sometimes a granular deposit, sometimes a general turbidity. On neutral litmus glucose-agar a blue colour is developed, indicating the production of alkalinity. Cultivated in peptone water an indole-like reaction with sulphuric acid alone can be obtained after a variable time, three to four weeks, while the diphtheria bacillus gives it in about a week; with a nitrite and sulphuric acid the indole-like reaction can be obtained with both the pseudo- and diphtheria bacilli in about a week. The substance giving this indole-like reaction is not indole, but skatole-carboxylic acid.² A broth culture reduces a weak solution of methylene blue. The bacillus does not curdle milk or liquefy gelatin, can be cultivated at from 22° to 37° C., and is non-pathogenic to guinea-pigs in doses of 5 c.c. or more of a forty-eight hours' broth culture. Some of the differences between the Hofmann bacillus and the Klebs-Löffler bacillus are shown in the table on the next page. In addition, the Hofmann bacillus does not ferment any sugar &c. (see Table, p. 268).

The histories of several cases investigated by Miss Knight and Hewlett seemed to show that the Hofmann bacillus is associated with mild anginal conditions, which

¹ Ordinary potato rendered alkaline with a 10 per cent. solution of sodium carbonate before sterilization.

² Hewlett, *Trans. Path. Soc. Lond.* li., 1900, p. 187; lii., 1901, p. 113.

Differences between the Klebs-Löffler and the Hofmann Bacillus.

	Hofmann Bacillus	Klebs-Löffler Bacillus
Morphology . . .	Rods 1.5 μ to 2 μ in length, tending to be slightly thicker at the centre than at the ends. Is 'plumper,' shorter, and less variable than the Klebs-Löffler bacillus	Rods averaging 3 μ to 4 μ in length. Slender and (excluding involution forms) of more uniform diameter than the pseudo. Considerable variation in size
	Involution forms rare	Involution forms usually present
Staining with Löffler's blue	Stains more deeply and regularly than the Klebs-Löffler bacillus. Polar staining rare	Staining generally more or less irregular, and polar staining common
Neisser's stain . . .	Negative	Positive
Alkaline potato . . .	Distinct cream coloured colonies or growth visible in two days	Grows well, but growth is almost invisible
Neutral litmus agar . .	Alkaline reaction	Acid reaction
Litmus milk . . .	Alkaline reaction	Acid reaction
Stab - cultures in glucose-agar and gelatin	Growth only at upper part of stab	Growth along whole length of stab
Anaërobic cultures in hydrogen	No growth . . .	Grows well
Indole-like reaction. (Peptone - water cultures, with sulphuric acid alone)	Only after three weeks' growth. (Due to skatole-carboxylic acid)	After one week's growth. (Due to skatole-carboxylic acid)
Fermentation reactions	See table on page 268.	

are free from complications, end in recovery, and are not followed by sequelæ. In many of the cases the anginal condition was associated with distinct patches of membrane, and in two, symptoms were present suggestive of the toxæmia which is met with in diphtheria.

In a long series of experiments Hewlett and Miss Knight believed that some evidence was obtained of the conversion of the Hofmann into the Klebs-Löffler bacillus and *vice versâ*. Moreover, the Hofmann bacillus seemed

in many instances to replace the Klebs-Löffler bacillus in the throat during convalescence, and it is possible in a large series of cultures to obtain connecting links between the Klebs-Löffler bacillus on the one hand, and the Hofmann bacillus on the other. Cobbett,¹ however, suggests that these facts are capable of another explanation, viz. that during the acute stage, diphtheria bacilli being readily found, the Hofmann bacillus is likely to be overlooked, whereas at a later stage a more careful search may be necessary to detect the diphtheria bacillus, and in the course of that search the Hofmann bacillus is therefore more frequently found.

Miss Knight and Hewlett came to the conclusion that in some cases, at least, the Hofmann bacillus is a modified Klebs-Löffler bacillus, and the view taken of its relation to the Klebs-Löffler bacillus was, that it is a very attenuated Klebs-Löffler bacillus, i.e. one far removed from virulence. It would therefore seem wise to treat anginal cases in which the pseudo-diphtheria bacillus is found as possibly infective, though it would probably be inexpedient to admit to a general diphtheria ward (unless a prophylactic dose of antitoxin be given), nor would antitoxin be needed in the majority.

Most authorities have been unable to convert the pseudo-bacillus into a virulent Klebs-Löffler bacillus or *vice versa*, and many are of opinion that it has probably nothing to do with diphtheria (Park and Beebe, Peters, Washbourn, Cobbett). A few fatal cases have been recorded (e.g. by Stanley Kent) in which a careful search has failed to reveal any but Hofmann bacilli. Boycott² found that the seasonal prevalence of the Klebs-Löffler and Hofmann bacilli does not correspond, the former prevailing during September, October, and November, the latter is more frequent from May to August.

¹ *Journ. of Hygiene*, i., 1901.

² *Journ. of Hygiene*, 1905, v. p. 223.

Salter¹ in a critical review of the relationship between the Klebs-Löffler and the Hofmann bacillus found that the latter was virulent to many small birds (goldfinch, chaffinch, canary, &c.), and that by successive passages it became converted morphologically into a Klebs-Löffler form with feeble virulence for the guinea-pig. He also found that the filtered broth culture of the Hofmann bacillus, though harmless to guinea-pigs, was toxic to small birds, and moreover that it contained a non-toxic substance (toxoid) which had the power of combining with, and neutralizing, diphtheria antitoxin. Salter concluded, therefore, that diphtheritic organisms are to be met with of every grade of virulence, the weakest, known as Hofmann's or the pseudo-diphtheria bacillus, representing the most attenuated form of the Klebs-Löffler bacillus. The writer,² Cobbett,³ Petrie,⁴ and Williams⁵ have, however, quite failed to confirm Salter's results.

To sum up: the Klebs-Löffler-like avirulent bacilli met with in the throat, the pseudo-diphtheria bacilli of Roux and Yersin, are probably modified and avirulent diphtheria bacilli. As regards the Hofmann bacillus, this may in reality include several species, of which one may be a modified Klebs-Löffler bacillus, the others having no relation with this organism. On this view the Klebs-Löffler-like avirulent bacilli might be regarded as true diphtheria bacilli *slightly* removed from virulence, the Hofmann bacillus, if derived from the Klebs-Löffler, as a diphtheria bacillus *far* removed from virulence.

Priestley records an outbreak of what he terms 'pseudo-diphtheria,' in which the Hofmann bacillus seemed to be the causative organism, and expresses the opinion that this bacillus is not related to the Klebs-Löffler bacillus.⁶

¹ *Trans. Jenner Inst. Prev. Med.* ii. p. 113. (Bibliog.)

² *Brit. Med. Journ.*, Sup. July 9, 1904.

³ *Journ. of State Med.* xi. p. 609.

⁴ *Journ. Med. Research*, 1902, p. 83.

⁵ *Journ. of Hygiene*, v. p. 134.

⁶ *Public Health*, July 1903.

In determining the fermentation reactions of the diphtheria-like bacilli, the organisms should first be grown in broth until they become acclimatised to this medium, or should be grown in a medium which suits them, e.g. broth with the addition of serum or of ascitic fluid. Hiss's serum-water medium is satisfactory—serum 1 part, water 3 parts, with 1 per cent. of the carbohydrate or other substance, tinged with litmus and sterilized in the steamer on three consecutive days. Graham-Smith¹ gives the following table of fermentation tests:

Organism	Hiss's Medium (10 days' growth)								
	Glucose	Lactose	Sucrose	Galactose	Maltose	Laeulose	Mannitol	Dextrin	Glycerin
<i>B. diphtheria</i> , virulent and avirulent	C A	C A	0	C A	C A	C A	0	C A	C A
Hofmann bacillus *	0	0	0	0	0	0	0	0	0
Xerosis bacillus *	C A	0	0	0	0	C A	0	0	C A
<i>B. coryza</i> *	C A	0	0	C A	0	C A	0	0	0
Diphtheria-like bacilli:									
from the ear *	0	0	0	0	0	0	0	0	0
from the urethra *	- A	0	0	- A	- A	- A	0	0	0
from the throat *	C A	0	0	C A	- A	C A	0	0	0
from the fowl *	- A	0	0	- A	- A	...	0	0	0
(* avirulent to the guinea-pig)									

C = coagulation; - = no coagulation; A = acid; 0 = no reaction. Slight variations were occasionally noted: for example, four out of twenty diphtheria bacilli gave no acid with lactose, and the amount of acid production and of coagulation was somewhat variable.

CLINICAL DIAGNOSIS.

(A) *In Man and Animals*:—I. In a minority of cases the diphtheria bacillus can be identified in the membrane or discharge, and the diagnosis established thereby.

¹ *Journ. of Hygiene*, vi. 1906, p. 286.

A fragment of the membrane is teased up as finely as possible on a slide, a droplet of water being added if necessary, and a couple of smear specimens are prepared. One of these should be stained with Löffler's methylene blue, the other by Gram's method. The bacilli will be found lying parallel to one another in larger or smaller groups, together with involution forms. Neisser's stain (see below) may also be of assistance. Higley of New York recommends staining the smears for five seconds in dilute carbol-methylene blue (7 drops to 10 c.c. water), rinsing and drying, and counter-staining in dilute carbol-fuchsin (10 drops to 10 c.c. water) for one minute, rinsing and drying.

II. Frequently the membrane is so crowded with different forms of organisms that it is extremely difficult to recognize the diphtheria bacilli with any degree of certainty. Recourse must then be had to cultivation.

For this purpose sloping blood-serum tubes, or tubes of serum-agar, must be employed; simple agar is unsuitable.

A piece of membrane or a swabbing from the throat is rubbed over the surface of one or two serum tubes, care being taken not to break up the medium. The tubes are then incubated at 37° C. for eighteen to twenty hours, and are then examined microscopically whether there is any visible growth or not. If there is no visible growth a scraping is taken by means of a sterilized platinum needle from the whole surface, and a cover-glass specimen prepared with a droplet of water. If there is a visible growth, the cover-glass specimen should be prepared from the most likely colonies, or, if the growth be confluent, from the upper half-inch or so. A microscopical examination must always be made, for some colonies—certain staphylococci and torulae, for example—simulate those of the diphtheria bacillus very closely. The cover-glass specimens should be stained with Löffler's methylene blue for five to ten minutes, washed, dried, and mounted in Canada balsam or cedar oil. If there is sufficient growth on the tubes, the preparations may be made on a slide, and after staining, washing, and drying, a drop of cedar oil may be put on the stained patch, which is then examined directly without a cover-glass. If, however, there is very little growth, it is better to make a cover-glass specimen, as the position of the material is so

much more easily located. The preparations are examined with a $\frac{1}{2}$ -inch oil immersion magnifying not less than 800-1000 diameters, and the Klebs-Löffler bacillus identified from the description given above.

Prausnitz considers that if negative results are obtained with eighteen to twenty-four hours' incubation, the tubes should be incubated for a further twenty to twenty-four hours and re-examined.

Löffler's methylene blue gives much more characteristic preparations than Gram's method.

Although eighteen to twenty hours is recommended for incubating the cultures, a microscopical examination will sometimes reveal the bacilli at a much earlier period. The writer has found them in as short a time as six hours, but if bacilli are then not found, the tubes must be incubated for the longer period.

Neisser¹ has recommended the following method of staining:

(a) One gram of methylene blue (Grubler's) is dissolved in 20 c.c. of 96 per cent. alcohol, which is then mixed with 950 c.c. of distilled water, and 50 c.c. of glacial acetic acid.

(b) Two grams of Bismarck brown are dissolved in one litre of boiling distilled water and the solution is filtered.

The preparations are stained in (a) for one to three seconds, rinsed in water, and stained in (b) for three to five seconds, washed in water, dried, and mounted. The bacilli are stained brown, and contain two, or rarely three, inky-blue dots. This is a valuable confirmatory stain for the diphtheria bacillus, but staining for a longer time than that recommended by Neisser is advisable, viz. half a minute in the blue and one minute in the brown.² Tanner treats with Gram's iodine solution for half a minute after the blue. The staining solutions seem to keep well but occasionally fail to act, so should be controlled on an undoubted diphtheria culture.

In the majority of cases, after a little experience, the Klebs-Löffler bacillus will be readily recognized if present. Occasionally, however, bacilli may be present which resemble the Klebs-Löffler very closely, and of which it is difficult to be certain. In such

¹ *Zeitschr. f. Hyg.* xxiv., 1897, No. 3, p. 443.

² Hewlett, *Trans. Jenner Inst. Prev. Med.* ii. p. 201. See also *Brit. Med. Journ.*, 1901, ii. pp. 758 and 1016.

a case the following points should be noted in attempting to come to a decision :

1. The character of the growth on the medium.
2. The depth of staining with Löffler's blue, and the presence or absence of segmentation or polar staining. The Klebs-Löffler bacillus usually stains somewhat deeply, while the bacilli resembling it stain but feebly.
3. The presence or absence of involution forms, clubbing, &c.
4. The presence or absence of thread forms. The Klebs-Löffler bacillus does not form threads.¹
5. The presence or absence of spores. The Klebs-Löffler bacillus does not form spores.
6. Motility in a hanging drop. The Klebs-Löffler bacillus is non-motile.
7. Gram's method of staining. The Klebs-Löffler bacillus stains well.
8. The grouping of the organism. The parallel grouping of the Klebs-Löffler bacillus is somewhat characteristic. The bacilli when lying side by side do not seem to quite touch, while the bacilli which resemble the Klebs-Löffler and show a parallel grouping frequently lie much closer together than the Klebs-Löffler bacillus ever does.
9. The reaction with Neisser's stain (*the culture must be a young serum one*). The pseudo-bacillus and other bacilli do not give the diphtheritic reaction (polar staining).
10. The final test of virulence may be applied. For this purpose the organism must be isolated in pure culture by plate cultivations. Two guinea-pigs, of 250-300 grams weight, are each inoculated with 2 c.c. of a forty-eight hours' broth culture, one receiving at the same time 1 c.c. of diphtheria antitoxin. If the guinea-pig inoculated with culture only dies, while the one receiving culture and antitoxin lives, this is complete proof that the organism is the diphtheria bacillus; if both live no inference can be made except that the organism is non-virulent; if both die, it shows that the organism is virulent, but that it is not

¹ Klein and others have described thread and branched forms in cultures of the Klebs-Löffler bacillus in certain circumstances, but these are not likely to be observed under the conditions mentioned.

neutralized by antitoxin, and therefore is not the diphtheria bacillus.

11. Agglutination tests are unsatisfactory and not of service.

It occasionally, though very rarely, happens that a conclusion cannot be arrived at without an extended investigation.

If serum tubes are not available an egg may be used. It is boiled hard, the shell chipped away from one end with a knife sterilized by heating, and the inoculation made on the exposed white; the egg is then placed, inoculated end down, in a wine-glass of such a size that it rests on the rim and does not touch the bottom. A few drops of water may with advantage be put at the bottom of the glass to keep the egg-white moist. The preparation is kept in a warm place for twenty-four to forty-eight hours and then examined. Antitoxin itself may be used as a culture medium. A test-tube is sterilized by heating, or with boiling water or steam from a kettle, antitoxin to the depth of about an inch is poured in, and is coagulated by holding the tube very obliquely in boiling water or steam. After coagulation and cooling the medium is inoculated. (N.B.—An antiseptic is frequently present in antitoxin.) If no incubator is available, the culture may be kept in a warm place, or in an inside pocket.

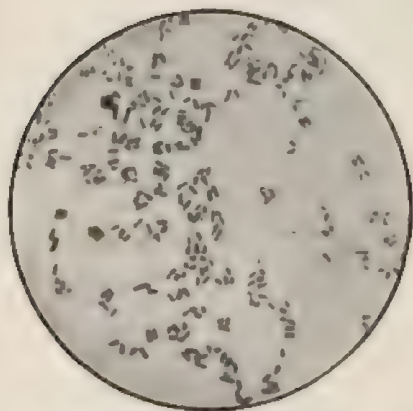
Many laboratories will now undertake the examination of material. Culture outfits are supplied by some, consisting of a sterilized tube containing a sterilized swab. Failing this, a piece of membrane may be forwarded in a tube or bottle which has been sterilized by heating, or with boiling water or steam. If there be no membrane, a swab can be readily extemporized by wrapping a little wool round the end of a piece of wire, knitting-needle, hair-pin, penholder, or splinter of wood. The wool may be sterilized by moistening with water and then holding in a flame. Membrane or secretion may also be forwarded on pledgets of wool, pieces of lint or calico, and even on paper, but these are not so suitable.

(B) *In Milk*.—See section on Milk.

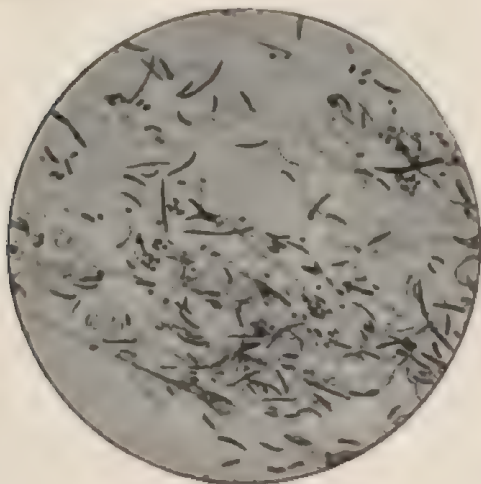
VINCENT'S ANGINA.

An infective malady characterized by sore-throat, fetor dysphagia, and ulceration and membrane simulating diphtheria.

PLATE VII.



a. THE PSEUDO-DIPHTHERIA OR HOFMANN BACILLUS.
COVER-GLASS PREPARATION OF A SERUM CULTURE. $\times 1500$.



b. VINCENT'S ANGINA.
SMEAR FROM EXUDATION SHOWING FUSIFORM BACILLI (DARK) AND
SPIRILLA (LIGHT). $\times 2000$.



The diphtheria bacillus, however, is not present, and according to Vincent¹ the affection is caused by a symbiotic association of a bacillus and a spirillum. The bacillus (*B. fusiformis*) measures 6-8 μ to 10-12 μ in length, has *pointed* ends, is often motile, and does not stain by Gram. It can be cultivated on the ordinary media to which human blood serum, ascitic or hydrocele fluid has been added. The spirillum is long and sinuous and very motile, but cannot be cultivated. The two organisms will be found on microscopical examination of stained smears of the secretion from the throat (Plate VII., b).

THE XEROSIS BACILLUS.

The xerosis bacillus was isolated by Neisser from cases of xerosis conjunctivæ, and is met with in follicular conjunctivitis. Lawson isolated it on many occasions from the normal conjunctival secretion. In morphology and staining reactions it resembles the Klebs-Löffler bacillus very closely. It differs from the Klebs-Löffler bacillus in the following particulars: (1) usually, but not always, in the *primary* cultivations from the eye on blood serum, colonies do not appear under about thirty hours, while those of the Klebs-Löffler bacillus are visible in sixteen to twenty hours. This does not apply to the *secondary* cultivations, in which the colonies appear as soon as those of the Klebs-Löffler bacillus. (2) Upon agar it will seldom or never grow in primary culture, and in secondary cultures it forms only a thin translucent *dry* film. (3) Upon gelatin it will never grow in primary culture and seldom in secondary culture. (4) It does not give rise to acid production in milk or glucose broth. (5) It is non-pathogenic to guinea-pigs. (6) The Neisser stain is negative. The fermentation reactions will be found in the table on p. 268.

In all probability the organism is not causative of xerosis conjunctivæ.

Griffith² found the xerosis bacillus in 120 out of 210 normal conjunctival sacs.

¹ See *Lancet*, 1905, i. p. 1260. (Bibliog.)

² *Thompson Yates Lab. Rep.* iv. Pt. i. 1901, p. 99.

To isolate the organism blood-serum tubes are inoculated with a looped platinum needle from cases of follicular conjunctivitis or xerosis, and incubated at 37° C. for forty to forty-eight hours. Half the tubes will usually show a growth. Preparations may be stained with Löffler's blue and by Gram's method.

BACILLUS CORYZE (SEGMENTOSUS).

An organism first described by Cautley, of frequent occurrence in the nasal secretion in cases of 'influenza' cold. It bears a striking resemblance morphologically to the *B. diphtheriæ* when stained with methylene blue, and is Gram-positive, but does not show granules either with Löffler blue or with Neisser's stain. On agar it grows more slowly than *B. diphtheriæ*, and in glucose broth and litmus milk acid production is slow and feeble. It is non-pathogenic to guinea-pigs. The fermentation reactions will be found in the table on p. 268.

OTHER DIPHTHERIA-LIKE BACILLI.

As already mentioned, diphtheria-like bacilli are not infrequent in wounds, pathological discharges and secretions. Some of them may be positive with Neisser's stain. They are always non-virulent. The fermentation reactions of some of these organisms will be found in the table on p. 268.

BACILLUS DIPHTHERIÆ COLUMBARUM.

Pigeon diphtheria, an infectious disease of pigeons, characterized by the formation of diphtheritic-like membranes on the tongue, fauces, and corners of the mouth, was described by Löffler, who isolated a bacillus to which he gave this name. It is short with rounded ends, non-motile, does not form spores, and does not stain by Gram's method. On gelatin it forms a whitish growth without liquefaction, on agar a creamy growth, and on potato a thin grey film. Milk is not curdled and is unchanged in reaction. It is pathogenic for the mouse and pigeon, but only slightly so for the fowl and guinea-pig. It is possible to prepare a vaccine, and an anti-serum for the disease.¹

¹ See *Ann. de l'Inst. Pasteur*, xv., 1901, p. 952.

Diphtheritic roup of poultry is a different disease, and is stated to be due to a protozoan parasite.¹ Macfadyen and the writer² have found Klebs-Löffler-like organisms to be present in the mouths and throats of healthy pigeons and fowls. These organisms resembled the true Klebs-Löffler bacillus in their cultural reactions, but were quite non-virulent to guinea-pigs.

The so-called diphtheria of calves is produced by an anaerobic streptothrix.

¹ See also Gordon Sharp, *Lancet*, 1900, ii. p. 18.

² *Trans. Path. Soc. Lond.* li., 1900, p. 13, and *Brit. Med. Journ.* 1900, i. p. 994.

MANUAL OF BACTERIOLOGY

CHAPTER IX.

ACID-FAST' BACILLI—TUBERCULOSIS—LEPROSY--
THE SMEGMA BACILLUS--GLANDERS.

'ACID-FAST' BACILLI.

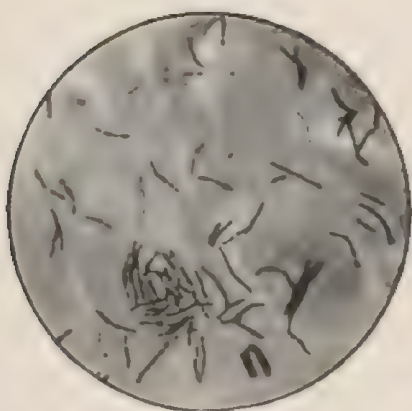
Important characteristic of the tubercle, leprosy, and
bacilli is the property they possess when stained
with carbol fuchsin of retaining the red colour after treatment
with strong mineral acid (25 per cent. sulphuric or
5 per cent. nitric). They are therefore termed 'acid-fast.'
Other organisms are rapidly decolorized even by
5 per cent. sulphuric acid, but it must be recognized
that several apparently saprophytic bacilli are also 'acid-

PLATE VIII.



a. THE TUBERCLE BACILLUS.

COVER-GLASS PREPARATION OF A PURE CULTURE. $\times 1000$.



b. TUBERCLE BACILLI IN SPUTUM. $\times 1500$.



'Mist bacillus'). It has been suggested that these saprophytic acid-fast bacilli may be derived from the tubercle bacillus, but Panisset's work gives no confirmation of this.

The *Streptotricheæ* occasionally exhibit 'acid-fast' properties. Dean has found acid-fast leprosy like bacilli in rats (see p. 313). All the acid-fast bacilli seem to be Gram-positive.

On 'acid-fast bacilli' see *Le Progrès Medical*, 1900, Dec. 1, p. 429 (Bibliog.); *Trans. Congress on Tuberculosis, London 1901*; *Journ. Exp. Med.* V. No. 2, 1900; Coles, *Journ. State Med.* xii., 1904; Panisset, *Rev. Gen. de Med. Vet.*, 1907, p. 533.

TUBERCULOSIS.

Tuberculosis is, unfortunately, only too common in the human subject, and most of the domestic animals and wild animals in a state of captivity suffer more or less from it.

The conception of tuberculosis was originally a purely anatomical one, the name being given to a condition in which the organs were studded with little yellowish points or nodules, which were termed tubercles. Laennec was the first to point out the characters of these nodules or tubercles, and traced with considerable accuracy their development from beginnings of the size of hemp seed, the miliary tubercles, up to the large cheesy masses which may be met with in the glands and lungs.

Microscopically, the structure of a young and typical tubercle is characteristic. At the centre one or more large nucleated protoplasmic masses are found, which on account of their size are termed giant cells, each containing ten or twenty nuclei situated at the periphery. These seem to be of the nature of plasmodia, similar to the masses of fused cells which surround a foreign body in the lower animals (Adami). Encircling these is a zone of cells possessing large and distinct nuclei, surrounded by a considerable mass of protoplasm and staining clearly, which, from their resemblance to epithelial cells, are known as

epithelioid, or more properly endothelioid, cells. Outside this again is another zone of smaller cells with scanty protoplasm and small nuclei, known as lymphoid cells from their likeness to the cells of lymphoid tissue. This is the structure of a typical tubercle, but one or other of the components may be wanting, and none can be said to be absolutely characteristic of the tubercle. The nodule possesses no blood-vessels, and as its size increases by growth at the periphery the central parts undergo degenerative changes, and may become either structureless or hyaline, or be converted into a soft yellowish material somewhat like cheese and termed caseous. Inflammatory reaction more or less extensive ensues in the tissues surrounding the tubercle, and the cellular elements so produced often become spindle-shaped and ultimately fibrous, so that the tuberculous nodule becomes enclosed by a capsule of fibrous tissue which may contract and convert it into a fibrous nodule. After caseation has occurred calcification may ensue—that is, lime salts are deposited and the nodule is converted into a calcareous mass.

As far back as 1865 Villemin showed that inoculation of rabbits with human caseous material was followed by a development of nodules similar in all respects to the miliary tubercles in man. Cohnheim, Burdon Sanderson, and Wilson Fox confirmed this observation, but they also showed that the development of tubercles apparently followed not only the introduction of tuberculous material, but also that of setons, pieces of putrid muscle, and gutta-serena. It was pointed out, however, that in all probability these results were due to accidental contamination or inoculation with tuberculous matter, and, by adopting suitable precautions in order to prevent such sources of error, it has been conclusively shown that non-tuberculous matter is unable to set up tuberculosis. Tuberculosis is therefore inoculable, and is an infective disease, and as such must

be due to a specific infective agent, to the discovery of which observers then turned their attention. In 1882 Koch announced that he had discovered a special bacillus, the tubercle bacillus, in tuberculous tissues, which could be isolated and cultivated, and which reproduced the disease on inoculation.

THE TUBERCLE BACILLUS.

Morphology.—The tubercle bacillus (*B. tuberculosis*) is a slender rod with rounded ends, often slightly curved, and measuring 3-5 μ in length. In stained preparations one or more unstained intervals are often seen in the rods (Plate VIII., *a*); these have been considered by some observers to be spores, but there are many objections to this view. Spores are usually single and not multiple, and are regular spherical or ovoid bodies, whereas the unstained spaces in the tubercle rods are irregular. Moreover, in the same specimen of sputum a varying amount of 'beading,' as it is termed, may be brought out by different staining methods (Plate VIII., *b*), in a preparation stained by Gram's method it is usually more pronounced than in one stained with carbol-fuchsin. In class work also it will be found that one student's specimen will show beading much more markedly than another's. These considerations render it probable that the beading is partly due to segmentation of the protoplasm, and partly perhaps is an artifact due to the staining process, and is not a spore formation. The tubercle bacillus, however, probably does form spores, though this is a debated point. Some observers have described clear, regular, unstained spaces in bacilli from old cultivations, and consider these to be true spores.

The tubercle bacillus is a non-motile, strictly parasitic organism (it has been described as being both motile and flagellated). It usually occurs singly, occasionally linked in twos or threes so as to form short chains, and under certain conditions, especially in old cultures, filamentous

forms develop. The bacillus is agglutinated by the blood serum of a tuberculous animal (see below, p. 305).

Staining Reactions.—The tubercle bacillus stains indifferently with the ordinary watery solutions of dyes, prolonged treatment with, or warming, the solution being required. It stains well by Gram's method. It also stains well and deeply with carbol-fuchsin, particularly on warming, and when so stained is markedly resistant to the decolorizing action of 25–30 per cent. mineral acid—that is to say, it is strongly 'acid-fast,' and this property is made use of for demonstrating its presence in tissues &c., and for diagnostic purposes.

Koch states that the peculiar staining reaction of the tubercle bacillus is due to a coating of two fatty acids, which take up the stain and are not decolorized by the mineral acid. De Schweinitz and Dorset (*loc. cit.* p. 286) have found the fatty substances to be principally a glyceride of palmitic acid, together with small amounts of lauric acid and of two other undetermined acids. Bulloch and Macleod (*loc. cit.* p. 276) found that the fat is not acid-fast, and by saponification yields oleic, isocetic, and myristinic acids. The acid-fast substance, according to these observers, is an alcohol.

Cultural Characters.—The tubercle bacillus is aerobic and facultatively anaerobic, and thrives best at a temperature of 37° C. or thereabouts, and development even then is slow, six weeks at least being required for an appreciable growth. Koch was unable to cultivate it except on blood serum, but Roux and Nocard afterwards found that it grows well on nutrient agar or veal broth to which 5 or 6 per cent. of glycerin have been added. The best method of isolating the bacillus from the tissues is to make use of Roux's potato tube (fig. 8), the bulb being filled with 5 per cent. glycerin. The potato is inoculated with an emulsion of the tuberculous organs and incubated at 37° C. In six or eight weeks

cultures will be obtained in half the tubes, whereas if serum or glycerin agar be used not more than 1 or 2 per cent. of the tubes will show a growth. The bacillus will also grow, though very slowly, on glycerin gelatin at 22° C. and on potato. The gelatin and blood serum are not liquefied. On glycerin agar the growth is characteristic: it forms a dry, crinkled and wrinkled, cream-coloured or brownish-yellow film, which has been well described as resembling the patches of lichen met with on trees (fig. 36). The growth, however, varies considerably both in colour and in the amount of wrinkling, though retaining more or less the characteristics just mentioned. In broth it forms soft cream-coloured, flocculent masses, which increase slowly both in size and number, the broth remaining perfectly bright and clear. Sometimes a dry crinkled film forms on the surface of the broth, and may spread all over it, and tends to creep up the sides of the vessel. This film formation seems to be essential for the preparation of a satisfactory tuberculin, but it is necessary in order to start it that some of the inoculated particles should float and form nuclei from which the film spreads. The virulent organism from the primary cultivations is difficult to grow on anything but glycerin potato.

Tuberculin.—Tuberculin is of two kinds, the *old* and the *new*; they are quite different preparations and must be distinguished from each other.



FIG. 36. —TUBERCLE BACILLUS.
GLYCERIN-AGAR CULTURE
THREE MONTHS OLD.

The *old tuberculin* is prepared by growing the tubercle bacillus for six to twelve weeks in a glycerin veal broth in a shallow layer in flat flasks (fig. 37), so that there is a free supply of oxygen and an abundant growth with copious film formation. The latter seems to be essential, but it does not appear to matter whether the bacilli be virulent or non-virulent, or whether they be of human or of mammalian origin. The cultures, bacilli and all, are concentrated over a water-bath to about one-tenth of their volume and then filtered through porous porcelain; the resulting fluid is thick, owing to the concentration of the glycerin by

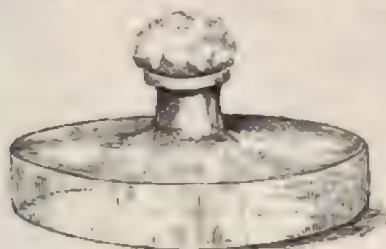


FIG. 37.—FLASK FOR GROWING TUBERCULIN.

the evaporation, is of a dark amber colour, and possesses a curious characteristic smell. The large proportion of glycerin preserves the fluid, which keeps indefinitely in a cool dark place.

This old tuberculin possesses remarkable properties. Injected into a healthy animal or individual it produces no effect, but in a tuberculous one minute doses, 0.003 gram, give rise to a marked reaction - elevation of temperature with constitutional disturbance more or less severe, and swelling and tumefaction of tubercular lesions (glands, ulcers, &c.) By cautiously increasing the amount a toleration is gradually induced, so that large doses cause little or no disturbance. Under certain conditions the injections of tuberculin produce marked changes in the tuberculous

parts, leading to necrosis and exfoliation, with subsequent healthy reaction and repair; this is especially seen in cases of lupus. By continued injections a marvellous improvement results, so much so that a cure is apparently effected; but unfortunately when the tuberculin treatment is discontinued the scar usually breaks down and the disease returns. Nevertheless a few cases have remained permanently healed.

Healthy guinea-pigs bear considerable injections of tuberculin without harm; but if they be tuberculous, if the disease is advanced (eight to ten weeks after inoculation), doses of 0.01 gram produce death; if less advanced (four to five weeks after inoculation) a larger dose, 0.2 to 0.3 gram, is required; but 0.5 gram always proves fatal. The post-mortem appearances are congestion of the lymphatics and viscera, and dark red spots, from mere points to the size of a hemp seed, on the liver and spleen. These are due to enormous dilatation of the capillaries in the immediate neighbourhood of tubercular deposits, actual extravasations of blood being rarely found. The hæmorrhagic-like spots on the liver are almost pathognomonic of death from tuberculin.

Koch found that if old tuberculin be mixed with five times its volume of absolute alcohol a brown resinous mass is deposited, the active principle, however, being still in solution. With a larger amount of alcohol the active principle is precipitated with the resinous mass. The old tuberculin contains about 10 per cent. of solid matter; attempts have been made to standardize it by intra-cerebral injection, but without much success.

If the alcohol be added to the tuberculin in the proportion of 2 : 3, a white flocculent powder is precipitated, which can be purified by washing with 60 per cent. alcohol and finally with absolute alcohol and drying *in vacuo*. This powder seems to contain the active principle; it is soluble

in water, but the solution diminishes in activity with age. A solution in 50 per cent. glycerin keeps well for several months and can be exposed to very high temperatures (130° to 160° C.) without diminution of activity. The pure substance gives all the protein reactions. Picric acid causes a precipitate which dissolves on heating and reappears on cooling; nitric acid also gives a precipitate, but hydrochloric and sulphuric acids do not. It contains sulphur, and from its reactions and elementary analysis evidently belongs to the protein group, and is most nearly allied to the proteoses.¹

Hunter² examined the old tuberculin, with the following result. Native protein is absent. It gives the proteose reaction with nitric and picric acids, and also the biuret reaction. Ammonium sulphate completely precipitates the protein matter, therefore peptones are absent. The proteoses present are proto- and deuterio-albumose, with some hetero-albumose and occasionally a trace of dys-albumose. It also contains traces of mucin, glycerin, extractives, and colouring matter, salts and two alkaloidal bodies. Hunter concluded that the proteoses are the substances possessing remedial and inflammatory actions, while the alkaloid bodies are fever-producing and not essential to its remedial properties.

The dose to commence with should be not more than 0.001-0.002 c.c., dilutions being made with 0.5 per cent. carbolic solution, and the dose is repeated when all reaction has passed away and is increased gradually. For treatment, the use of the old tuberculin has almost completely been given up in favour of the new tuberculin TR.

The New Tuberculin.—In endeavouring to obtain substances having an immunizing action against the tubercle bacillus, Koch devised three new modifications of tuberculin, all prepared from young and *virulent* cultures, which

¹ *Lancet*, 1891, ii. p. 976.

² *Brit. Med. Journ.* 1891, ii. p. 169.

are dried *in vacuo* and triturated by machinery.¹ The first, termed TA (tuberculin, alkaline), is prepared by stirring and shaking tubercle bacilli with a 10 per cent. solution of caustic soda and filtering. This preparation produces much the same reaction and effects as the original tuberculin; but has an insuperable drawback—it produces abscesses at the seat of inoculation when injected in any quantity. The two other modifications, termed TO and TR, are prepared by thoroughly triturating tubercle bacilli, emulsifying with distilled water, and centrifugalizing. After centrifugalizing the emulsion forms two layers—an upper one consisting of a white opalescent yet translucent liquid free from bacilli, and a lower consisting of a muddy residue. The former is the tuberculin O or TO (O=*ober*, upper). The residue is collected, dried, triturated and centrifugalized, and again yields two similar layers. The same operation is repeated several times until no residue is left. These preparations do not give rise to abscesses, and the upper layer after the first centrifugalizing has alone to be distinguished from the subsequent ones, the liquids resulting from the second and succeeding centrifugalizations being all alike in their action; they form the tuberculin R or TR (R=*residual*).

It is the tuberculin R, or *new* tuberculin, which is now used for treatment; it contains 2 mgrm. of solid matter per cubic centimetre (not 10 mgrm. as formerly stated), and for use is diluted with 20 per cent. sterile glycerin solution.

Tuberculin O closely resembles tuberculin A, but does not cause suppuration; its immunizing properties are, however, feeble. Tuberculin R, on the contrary, possesses distinct immunizing properties and causes neither reaction nor suppuration. According to Koch, healthy guinea-pigs treated with increasing doses of TR become so completely

¹ *Deutsch. Med. Wochenschr.* 1897, April 1 (translations or abstracts in most of the medical journals of about this date).

immunized that they are unaffected by subsequent inoculation with virulent bacilli. In guinea-pigs first inoculated with virulent bacilli and then treated by injections of TR retrogressive changes in the infected organs are always met with, and provided the treatment be commenced within one to two weeks after inoculation, a cure is wrought. For treatment of tuberculosis in man the initial dose is equivalent to not more than $\frac{1}{1000000} - \frac{1}{200000}$ mgrm. of solid matter. The doses are given subcutaneously at intervals of 10-14 days, and the treatment should be controlled by opsonic determinations. According to Latham, tuberculin may also be given by the mouth. Cases of cutaneous or localized tuberculosis, and those in which the opsonic index to tubercle is moderately reduced, do best. In phthisis and visceral tuberculosis no striking results have been obtained.

Behring has prepared another tuberculin, tulase or TC, by treating tubercle bacilli with chloral, which he states has a marked curative action, and is better administered by the mouth than by sub-cutaneous inoculation. By giving tulase to cows, the milk is said to acquire immunizing and curative properties which are transmitted to those consuming it. Other tuberculins are also on the market.

Crookshank and Herroun¹ obtained from glycerin broth cultures of the tubercle bacillus a proteose and an alkaloidal body. The proteose was also obtained from 'perlsucht.' Both the alkaloid and the proteose (from both sources) produced a rise of temperature in tuberculous guinea-pigs, while in healthy animals the former caused a slight, and the latter a marked, fall in temperature.

De Schweinitz and Dorset² describe some chemical products they have isolated from the tubercle bacillus grown in a special glycerin-asparagin mixture. From the bacilli

¹ *Brit. Med. Journ.* 1891, i. p. 401.

² *Med. Journ. N.Y.*, 1897, July 24, p. 195. Also *Fifteenth Annual Rep. Bureau of Animal Industry, U.S.A.*, 1898.

themselves an acid body was isolated, probably teraëonic acid, an unsaturated acid of the fatty series. A certain amount of the same body was also obtained from the special culture medium, but only a trace from glycerin broth in which the bacilli had been cultivated; in the latter case not because it was not formed, but because of the difficulty of isolation. This acid seemed to produce on injection depression of temperature and necrosis of the tissues locally. It has also some immunizing power, and may be the substance producing caseation in the tubercular nodules. The bacilli extracted with hot water yielded an albuminoid, which gave the tuberculin reaction. This they regard as the fever-producing substance.

Maragliano¹ states that toxic bodies are present in the blood and urine of tuberculous individuals. Cellulose also seems to be present in small amount in the bacilli (it has also been found in tubercular nodules).

Tubercle bacilli, living or dead, are with great difficulty absorbed when in any quantity. The dead bacilli when injected under the skin invariably cause suppuration, and several months later it is still possible to detect in the pus numerous bacilli which stain well; introduced into the circulation of rabbits they give rise to nodules in the lungs similar to the tuberculous nodules produced by living bacilli (Koch).

Action of Heat and Antiseptics on the Tubercle Bacillus.—The thermal death-point of the bacillus has been the subject of some controversy. Sternberg found that tuberculous sputum exposed for ten minutes to a temperature of 90°, 80°, and 66° C. failed to infect guinea-pigs on inoculation, while another specimen of the same sputum heated for ten minutes to a temperature of 50° C. produced tuberculosis in a guinea-pig, so that from these experiments the thermal death-point lies between 50° and 66° C.

¹ *Zeitschr. f. Tuberk. u. Heilstättenwesen*, i. p. 11.

Yersin in 1888, by culture methods, failed to obtain any growth from bacilli which had been heated to 70° C. for ten minutes, while those heated to 55° C. and 60° C. gave growths in glycerin broth in ten days and twenty-two days respectively. Macfadyen and the writer, in the course of some experiments on the sterilization of milk, found that milk to which powdered dried sputum had been added was rendered innocuous by a momentary heating to 67°-68° C. These experiments indicate that a temperature of 65° C. and over is probably rapidly fatal to the tubercle bacillus, so that milk which has been pasteurized (i.e. heated to 68°-70° C. for twenty to thirty minutes) may be regarded as quite safe. Experiments by the Royal Commission on Tuberculosis with virulent tuberculous milk gave somewhat irregular results; in one instance heating to 65° C. for two and a half minutes rendered the milk innocuous, in another instance after five minutes at 70° C. it was slightly virulent, but twelve minutes at the same temperature rendered it inert (see also section on Milk).

The tubercle bacillus offers considerable resistance to the action of antiseptics and germicides. Yersin found that it was killed by 5 per cent. carbolic acid in thirty seconds, by 1 per cent. in one minute, by absolute alcohol in five minutes, and by mercuric chloride, 1-1000, in ten minutes. Crookshank found that tuberculous sputum mixed with an equal volume of 5 per cent. carbolic was rendered innocuous in a few minutes, and this without any special precautions as to breaking up the masses. For disinfecting sputum mercuric chloride is unsuitable. (See also Chap. XXI.)

Pathogenesis &c.—Man is, unfortunately, only too frequently attacked with tuberculosis, the manifestations of which tend to differ somewhat at different age periods. Thus, in the very young, general miliary tuberculosis, tubercular meningitis, and tubercular disease of the peritoneum, intestine, and mesenteric glands (tabes mesen-

terica) are the commonest; in older children, up to the age of puberty, tubercular disease of lymphatic glands, especially in the neck, tubercular disease of joints and bone, and tubercular disease of the skin in the form of lupus are the most common; in young adults, tubercular disease of the lung; and in older people, chronic disease of the lung and tubercular disease of the urinary organs and testes, and of the suprarenal capsules (Addison's disease). Scrofula and struma were terms formerly much employed: both denote a swollen neck and were applied to cases the subjects of chronic tubercular inflammation with enlargement of the lymphatic glands, the cervical glands being most often affected, and frequently associated with other conditions, such as inflammation of the ear, throat and eye, and implication of bones and joints.

The distribution of the bacillus in the tissues varies considerably. In young and active tubercles the bacilli are more plentiful and more easily demonstrated than in older and more chronic ones. They tend to be more numerous in some animals than in others—in the ox and horse than in man, for example. In man the bacillus is difficult to demonstrate (by staining) in enlarged and caseating glands, in pus, in synovial membranes, and in lupus. In some animals, especially the ox and horse, bacilli can usually be readily demonstrated and may be present in large numbers, and frequently have the typical distribution, viz. within and at the periphery of the giant cells, though they are by no means confined to this locality (fig. 38).

The majority of the domestic animals are subject to tuberculosis. It is most common in the ox, pig, and horse, much less so in the sheep and goat, cat and dog. Wild animals, both mammals and birds, in a state of captivity are also specially prone to be attacked, and a large number of the deaths at the Zoological Gardens, London, particularly among the apes, are due to this disease.

In carp, tubercle-like nodules are occasionally met with in which a bacillus resembling the tubercle bacillus in morphology and staining reactions is present. It grows, however, much more freely than the true tubercle bacillus, and though inoculable into fish and frogs, is non-inoculable into warm-blooded animals. But it yields a tuberculin

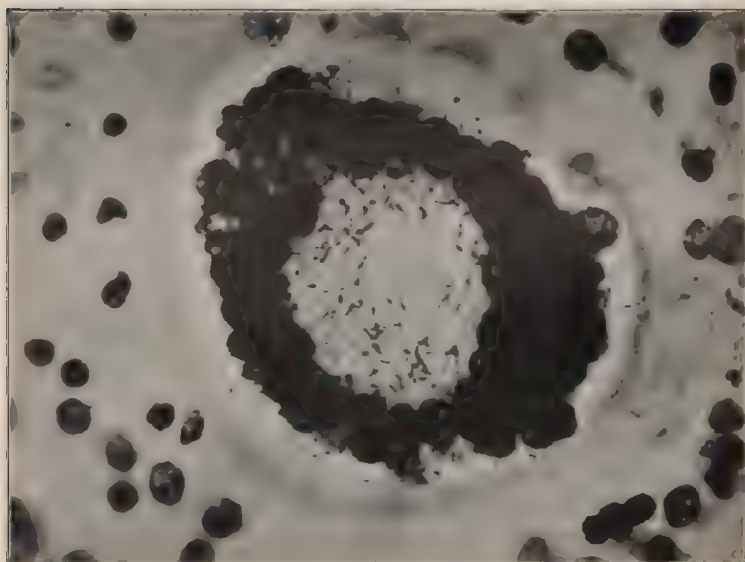


FIG. 38.—GIANT CELL CONTAINING TUBERCLE BACILLI. $\times 1000$.

which reacts with mammalian tuberculosis, and by feeding carp on the mammalian tubercle bacillus this can apparently be transformed into the piscian variety.

Bird or avian tuberculosis undoubtedly differs in many respects from mammalian tuberculosis. The tubercular new formations may be very large, but do not show nearly such a disposition to caseation or suppuration as the human lesions. Epithelioid cells form the major part of the

growth, and giant cells are very infrequent. One remarkable feature is the enormous numbers of bacilli which may be present in the tissues; in places they may be so numerous and closely packed as to form distinct masses or nodules. The bacilli of avian have the same staining reaction as those of mammalian tuberculosis, but on cultivation and inoculation various differences between the two races become evident.

The mammalian bacilli flourish best at about 37° C., and growth ceases at 41° C., whereas the avian bacilli thrive luxuriantly at 43° C., and the growth of the latter on glycerin agar is much moister and more wrinkled, and often more pigmented, than that of the former. Fowls and dogs are with difficulty infected with human bacilli, but dogs are susceptible to infection with avian bacilli. In all probability the avian bacillus is a variety of the mammalian and not a distinct species, as, by cultivation on boric-acid agar and on eggs &c., the mammalian bacilli assume the characters of the avian. The subject of avian tuberculosis is of some practical importance, as several cases are now recorded in which the bacilli cultivated from human cases were of the avian type, and probably derived from an avian source of infection.

Nocard described two types of tuberculosis in the horse, one in which the tuberculous lesions are chiefly abdominal, while in the other the lungs and bronchial glands are most severely affected. From a long series of observations he came to the conclusion that, generally speaking, the bacillus obtained from the pulmonary variety is of the ordinary mammalian type, while that of the abdominal one belongs to the avian.¹

As regards mammalian tuberculosis, Koch's address at the Congress on Tuberculosis² in 1901 upset our precon-

¹ *Journ. of Comp. Path. and Therapeut.* ix., 1896, p. 173.

² See *Brit. Med. Journ.* 1901, ii. p. 180.

ceived ideas. It had been known that there are certain differences between the bacilli of human and of bovine tuberculosis, the latter being shorter and thicker and more readily cultivated than the former; also while human tubercular material injected into a rabbit produces small discrete lesions in the organs, bovine material produces large caseating masses. These distinctions had been regarded as being due to variations in the bacilli as a result of growing upon a different soil and not to any fundamental difference between the two strains of bacilli. Koch, however, stated that young cattle and swine could not be infected with human tuberculous material, and he therefore concluded that human and mammalian tubercle bacilli are essentially different. As a result of his experiments he concluded that 'though the important question whether man is susceptible to bovine tuberculosis at all is not yet absolutely decided, if such a susceptibility really exists, the infection of human beings is but a very rare occurrence.'

This view met with considerable opposition, and a Royal Commission was appointed to investigate the question, and the following summarizes the results obtained up to the present, from which it will be gathered that there is no justification for assuming that man is infected from human sources alone. Thirty different viruses isolated from cases of tuberculosis occurring spontaneously in bovines have been studied, and the results of introducing them into a number of different animals by feeding and by inoculation are recorded. In calves, inoculation usually results in generalized progressive tuberculosis, but the effect is somewhat dependent on the dose, i.e. the number of bacilli, administered. Thus whereas 50 mgrm. of culture always produced a fatal generalized progressive tuberculosis, in two instances much smaller doses—0.01-0.02 mgrm.—produced only limited retrogressive tuberculosis. Feeding, on the other hand, usually produced lesions limited

to the neighbourhood of the digestive tract, which generally retrogress and become calcareous. The bovine bacillus, when introduced into rhesus monkeys or chimpanzees either by inoculation (even in so small a dose as 0.001 mgrm.) or by feeding, induces rapid generalized tuberculosis, and, considering the close relation that exists between the anthropoid apes and man, these results are of the highest importance. In pigs, generalized progressive tuberculosis is readily set up both by feeding with and by the inoculation of bovine bacilli. Goats, dogs, and cats are relatively less susceptible, but more or less tuberculous infection can similarly be produced in them. On this part of the investigation the Commissioners remark that the bacillus of bovine tuberculosis is not so constituted as to act on bovine tissues only, and the fact that it can readily infect the anthropoid apes, and, indeed, seems to produce this result more readily than in the bovine body itself, has an importance so obvious that it need not be dwelt on. The viruses isolated from sixty cases of the disease in man have also been studied, and the results obtained show that they may be divided into two groups, subsequently referred to as Group I. and Group II. The bacilli of Group I. comprise fourteen viruses, one obtained from sputum, three from tuberculous cervical glands, and ten from mesenteric glands of primary abdominal tuberculosis in children. The results produced by introducing these viruses into animals are identical with those produced by the bovine bacillus. The bacilli of Group II. comprised forty viruses obtained from various forms of human tuberculosis—cervical glands, mesenteric glands (8), lungs and bronchial glands (10), joint and bone disease (9), testes, kidney, &c.—grow more luxuriantly in culture than those of Group I., and inoculated into calves and rabbits do not produce the generalized and fatal disease caused by the bovine bacillus, but in rhesus monkeys and in the chim-

panzee set up a general tuberculosis. Certain human viruses, differing in certain respects from those of Groups I. and II., were also met with and are classed as Group III., but an opinion on their significance is reserved for a future report.

The Commissioners conclude that the tubercle bacillus in its nutritive and reproductive powers resembles other simple organisms, and that the essential difference between one strain and another depends on variations in these factors, and they therefore classify the bacilli as *dysgonic* those that grow with difficulty on artificial media, and as *eugonic* those that grow readily on these media.

The bearings of the results obtained are thus summarized :

'There can be no doubt that in a certain number of cases the tuberculosis occurring in the human subject, especially in children, is the direct result of the introduction into the human body of the bacillus of bovine tuberculosis, and that in the majority of these cases the disease is introduced through cow's milk. Our results clearly point to the necessity of measures more stringent than those at present enforced being taken to prevent the sale or the consumption of tuberculous milk.'

As regards the histological appearances of the tuberculous process in different animals Dr. Eastwood states that there is an underlying unity of the morbid processes produced experimentally by infection with every variety of bovine and human tubercle bacillus.

With regard to the channel of infection in human tuberculosis opinions differ. Koch insisted that inhalation of air-borne bacilli derived from dried human sputum is the principal source of infection ; Von Behring, on the other hand, has expressed the opinion that tuberculous milk fed to children is the main source of infection both of children and of adults ; in the latter case bacilli are ingested in

childhood and lie dormant for years before becoming active. At the Congress of Hygiene at Berlin, 1907, Flugge stated that his experiments showed that tuberculosis can be communicated to animals by inhalation, and that the dose of bacilli required to infect by the respiratory tract is very far less than that required to infect by the alimentary canal. The mode of infection in man doubtless varied, and he believed that children may be infected by the digestive tract, by tuberculous food, particularly milk, but the most extensive source of infection is the number of droplets of tuberculous expectoration coughed up by consumptives; these float in the air and serve as sources of infection to others.

Ribbert and Schrötter also, from the evidence of autopsies, considered inhalation as the chief mode of infection in man. Calmette, on the other hand, believes that in the young, infection by the digestive tract, especially by tuberculous milk, is the most frequent, and attaches little or no importance to dry dust containing tubercle bacilli as a source of infection. Ravenel considers that the alimentary tract is a frequent portal of entry, particularly in children, for the tubercle bacillus, which he believes is able to pass through an intact mucous membrane. Of sixty cases of human tuberculosis investigated by the Royal Commission on Tuberculosis twenty-eight possessed clinical histories indicating that in them the bacillus was introduced by the alimentary canal.

The occurrence of tuberculosis in the domestic animals raises some points of practical value, especially the occurrence of infection from the consumption of meat and milk from diseased animals. In the ox the tuberculous lesions are most frequently met with in the lymphatic glands and serous membranes, particularly the pleura, and in the lungs and liver, while the fat and muscular tissues, which constitute the major part of 'meat,' are very rarely affected.

On the pleura the growths take the form of nodular masses, which from their arrangement are popularly termed 'grapes' or 'angle berries.' There can be no doubt that the carcase of an animal extensively affected with tuberculosis, especially if wasting has occurred, should be condemned as unfit for food, and likewise all parts in which there are tubercular deposits. But it becomes an important question for the community, financially as well as from a hygienic point of view, as to the method of procedure with the meat from a beast comparatively slightly affected with tuberculosis—an enlarged gland or two, and a few nodules on the pleura. No doubt the ideal method is the condemnation and destruction of all tuberculous carcasses, be the amount of tubercle ever so little; but from a financial point of view this becomes almost an impossibility because of the large amount that would have to be paid in compensation. Experiment has demonstrated that the tubercle bacilli are practically confined to the tuberculous areas and are extremely rarely met with in the muscular tissue, and these portions, therefore, it might seem, could be eaten with impunity, especially as they would be cooked before consumption.

The Report of the first Royal Commission on Tuberculosis, however, indicates two dangers. Firstly, in cutting up a carcase the butcher will most likely use the same knife throughout, and in this way he may infect the meat with tuberculous matter by smearing with the knife. Secondly, cooking cannot be depended upon to destroy the bacilli unless the joints are under 6 lb. in weight; when the weight is above this the temperature in the interior may not rise sufficiently high. Evidently one of the first things to be done is the abolition of private slaughterhouses and the establishment of municipal abattoirs where the meat would have to be passed by competent inspectors. In this way all badly affected carcasses would be condemned, and those only

slightly affected could be separately dealt with and special precautions taken to eliminate tuberculous pieces &c.

Tuberculous milk also raises many important points. It is noteworthy that the incidence of abdominal tuberculosis in young children occurs just when cow's milk is the staple article of their diet. To avoid risk, all milk intended for the food of infants and young children should be pasteurized by heating to a temperature of from 68° to 70° C. for twenty minutes, or be sterilized by boiling.

Much might be done by the registration of all dairy premises and the elimination of all tuberculous animals from dairies, and by enforcing the inspection of dairy cattle by competent inspectors at intervals of not longer than a fortnight, making the notification of any disease of the udder compulsory, and the sale of milk from such a diseased udder illegal under a heavy penalty (Roy. Com. on Tuberculosis). The matter, however, presents considerable difficulty. The ideal method, and one which commends itself at first sight as being the most satisfactory, is the slaughter of all animals which are tuberculous. This was adopted in the State of Massachusetts; under an order of the Board of Cattle Commissioners all beasts in the State were tested with tuberculin, and every animal that reacted was slaughtered, and strict quarantine combined with the tuberculin test imposed on all imported cattle. Even in this small State such a plan was found to be unworkable, the expense being so heavy; and in Great Britain for such a system several millions would probably be required to settle the claims for compensation. A middle course will have to be adopted, viz. all manifestly tuberculous animals, especially where wasting or tubercular udder is present, to be slaughtered; animals to be tested with tuberculin, and those which react to be separated from the healthy and to be disposed of as soon as convenient, and in the meanwhile kept as much as possible in pasture.

Every available method should be adopted by local authorities and others to prevent the spread of tuberculosis. It can hardly be doubted that the disease, or at least phthisis, should be made notifiable, though there are many difficulties in carrying this out. Patients should be warned of the danger of disseminating their expectoration, and should use pocket spittoons containing an antiseptic, or handkerchiefs (such as the Japanese paper ones) which can be destroyed. Rooms which have been inhabited by tuberculous patients should be disinfected, for which purpose Delépine recommends spraying with a 1-100 solution of ehloride of lime. Although the occurrence of direct infection can rarely be proved, the possibility of this cannot be ignored.

Serum Therapeutics and Vaccine.—Maragliano's serum is prepared by injecting cows with watery tuberculin and with a bacillary pulp made by grinding up tubercle bacilli, emulsifying in water and filtering through a porcelain filter. Subcutaneous injections of the two preparations are given in increasing doses, commencing with 5 c.c. of each, until 20 c.c. is reached, the frequency of the injection being determined by the temperature reaction and general symptoms produced.

Marmorek's serum is prepared by growing tubercle bacilli in a medium consisting of a leucotoxic calf serum (prepared by injecting calves with leucocytes) and glycerin liver bouillon.¹ The filtered culture is injected into horses, and their serum, after several injections, becomes antitoxic to a slight degree. It cannot be said that even encouraging results have been obtained with these or other sera.

For *vaccine treatment*, tuberculin R is usually employed (p. 286). Latham has recently found that given *per os* it produces its characteristic effects.

Immunity.—Attempts have been made from time to

¹ *Bull. de l'Inst. Pasteur*, I., 1903, p. 851.

time to produce immunity against the *B. tuberculosis*, particularly in cattle. Thus McFadyean¹ found that heifers which had previously been subjected to repeated doses of tuberculin (old), in some cases resisted infection with virulent bacilli. Behring² has also employed human tubercle bacilli for the vaccination of cattle with satisfactory results. His tulase likewise confers immunity when given either by the mouth or by the stomach.

Theobald Smith³ also concludes that vaccination of calves with the human type of bacillus is harmless, and that the procedure leads to a relatively high resistance to fatal doses of the bovine bacillus.

CLINICAL EXAMINATION.

1. The 'complement-fixation' test has been tried in tuberculosis by Wassermann and Brück.⁴ Tuberculous anti-bodies, however, appear to be absent in cases of localized or latent tuberculosis, and the test cannot therefore be applied. But in acute miliary tuberculosis, Brück⁵ has found them present in the early stages.

2. The examination of sputum &c. for the tubercle bacillus is now a routine procedure and is often of the greatest value in forming a diagnosis. Fortunately, owing to the peculiar staining reaction of the tubercle bacillus, discovered by Koch, the method is comparatively simple.

1. *Sputum*.—Film specimens are prepared by smearing with a platinum needle a little of the sputum on a slide so as to form a thin film covering two thirds of the surface, or by placing a particle of the sputum on one slide, applying another slide, pressing together, and then drawing apart so that a thin film is left on each slide. The thick portion of the sputum should be used, the thin mucoid portion being rejected. If there are any small yellow caseous particles present, these should be chosen, and

¹ *Trans. Path. Soc. Lond.* liii. 1902, p. 20.

² *Brit. Med. Journ.* 1906, ii. p. 577.

³ *Journ. Med. Research*, XVIII. 1908, p. 451.

⁴ *Deut. Med. Woch.* 1906, xxxii. p. 449.

⁵ *Ibid.* p. 945.

sufficient material should be used so as to form a distinct but not too thick film; a little experience will soon decide the right amount. Preparations may also be made by smearing the sputum on a cover-glass or between two cover-glasses instead of using slides. Whichever plan is adopted, the film is dried and fixed in the usual manner (generally by heat) and then stained by one of the following methods:

(a) *Ziehl-Neelsen Method.*—Warm filtered carbol-fuchsin two to five minutes (one minute is often quite sufficient). Film specimens on slides are most conveniently stained by flooding with the dye and warming on a piece of asbestos cardboard supported on a tripod, or slides or cover-glasses flooded with the stain may be held in the forceps and carefully warmed over a flame, or the preparations may be immersed in a watch-glass or dish of the stain, covered, and placed in the warm incubator for half an hour. In no case must the stain be allowed to boil; it should only be warmed sufficiently to steam (50°–60° C.), and with slides or cover-glasses as evaporation takes place more stain (always filtered) should be added. After staining, the preparations are washed in water and then decolorized by treating with 25 per cent. sulphuric or 30 per cent. nitric acid. In decolorizing, when the preparation is immersed in the acid in a watch-glass or small beaker its colour changes to a yellowish brown, but on rinsing in water a good deal of the pink colour returns. The treatment with acid and with water alternately is repeated until the preparation is practically colourless when rinsed in water. With sputum this is usually the case after three or four rinses in the acid, but it varies with the thickness of the film and with the number of tubercle bacilli present; when these are absent the film often decolorizes more readily than when there are many. The presence of blood renders the decolorization difficult. After decolorizing and washing, the preparations are stained for one minute in Löffler's methylene blue, washed in water, and mounted in water, or, better, dried and mounted in Canada balsam or cedar oil. When the preparation is made on the slide, after washing and drying, it can be examined directly without a cover-glass with the oil-immersion after applying a drop of cedar oil, unless a permanent specimen is desired, in

which case a drop of balsam is put on and the film covered with a cover-glass.

The tubercle bacilli appear as delicate red rods, often beaded or segmented, on a blue background composed of cells, mucus, and putrefactive or other bacteria. Occasionally here and there a little red colour may be present in addition to the tubercle bacilli. Hair and keratinized material generally, such as horny epithelium, and red-blood corpuscles, retain the red colour after the foregoing treatment, and the spores of bacteria are also liable to retain the red somewhat persistently. These exceptions are not, however, likely to prove a source of error, for the tubercle bacilli should be recognised not only by their red colour, but also by their characteristic size, shape, and general appearance. It is conceivable that acid-fast bacilli not tubercle might be present in sputum, but such an event is a very unlikely one. For the microscopical examination, a $\frac{1}{4}$ -inch with good illumination is sufficient when the tubercle bacilli are present in any number. When they are scanty it is necessary to use a $\frac{1}{2}$ -inch oil-immersion, and this is the better lens in any case.

If tubercle bacilli are not found, other specimens should be prepared and examined. *It is only by repeated examinations on different occasions that the negative evidence, the absence of tubercle bacilli, becomes of any value.*

The tubercle bacillus is occasionally not acid-fast¹; probably the bacilli in such cases are degenerate and, like all degenerate bacteria, fail to stain well. In material which has been preserved a long time, e.g. sputum with carbolic, or tissue in spirit, the bacilli may be much less acid-fast than in fresh material.

Various methods have been recommended for the solution of the sputum and the examination of the sediment for the bacilli, after sedimentation or centrifugalizing, but they are more or less complicated and do not present any special advantage if several specimens are examined. (See section on Milk.)

If it is inconvenient to examine the sputum for a day or two a little 1-20 carbolic should be added. This preserves the sputum and the tubercle bacilli seem to retain their staining power unimpaired in such a mixture, certainly for many months.

¹ See *Lancet*, 1908, i. p. 1222.

If the tubercle bacillus cannot be detected microscopically after repeated examinations, and it is important to make a diagnosis, the inoculation method may be employed. A couple of guinea-pigs are inoculated subcutaneously in the thigh or abdomen with 0.5 to 1 c.c. of the sputum. If tubercle bacilli are present the animals will show signs of tuberculosis in three to six weeks (see below, Urine).

(b) *Gabbet's Method*.—Prepare and stain the specimens in carbol-fuchsin as in method (a). Then wash and treat with the following solution for two to three minutes; wash, dry, and mount:

Alcohol	50 parts
Water	30 parts
Nitric acid	20 parts

Saturate with methylene blue and filter.

This solution decolorizes and at the same time counter stains.

II. *Tissues*.—The histological appearance of the tubercle is usually sufficient for diagnostic purposes without the necessity of demonstrating the tubercle bacilli, which in many instances may be difficult in human material, as the bacilli may be very scanty, or practically impossible to demonstrate, e.g. in lupus. Sections should be prepared either by the freezing or the paraffin method, stained with hæmatoxylin, and counter-stained with eosin, or orange-rubin, or with the Ehrlich-Biondi mixture.

In order to demonstrate the tubercle bacillus in fresh tissue smears may be made and stained like sputum, or sections prepared and stained in warm carbol-fuchsin for about ten minutes. For frozen sections the stain may be contained in a watch-glass or small glass capsule, and is warmed until it steams, but not boiled, on a wire-gauze tripod or a sand-bath. Paraffin sections should be fixed to the slides with glycerin albumin, and may be stained by flooding with the carbol-fuchsin and warming on asbestos card-board for ten minutes. After staining, the sections are washed in water and are then decolorized in 25 per cent. sulphuric acid. This takes longer than with sputum, and the sections after being in the acid for a few seconds are washed in water and then returned to

the acid, and this alternate rinsing in acid and in water is repeated until they are colourless or nearly so when placed in water. It is not necessary to remove the colour absolutely; a faint pink remaining does not matter. After rinsing in fresh water to remove all the acid, the sections are counter-stained in Löffler's methylene blue for two minutes, rinsed in methylated spirit, passed through absolute alcohol somewhat rapidly to avoid removing too much of the blue, cleared in cedar oil or xylol, and mounted in balsam. The sections may also be counter-stained with hematoxylin or Bismarck brown.

Instead of using the strong acid solution for decolorizing an acid alcohol solution may be used with advantage.

Gram's method may also be used, but is, of course, not distinctive for the tubercle bacillus.

The following is a good method for staining tubercle in sections (Kühne, modified by Delépine¹):

1. The tissues should be fixed in corrosive sublimate, acidulated or not, hardened in alcohol, embedded in paraffin, and the sections fixed on slides with glycerin albumin.

2. The sections are stained with hamatin solution for ten to twenty seconds to obtain a pure nuclear stain (not too deep), then washed thoroughly in water.

3. They are then stained with carbol fuchsin, kept at a temperature of about 47° C. for twenty to thirty minutes. The slides are during that time kept in a moist chamber to prevent the stain from drying on the specimen.

4. The stain is rinsed off with water, and the sections are treated with 2 per cent. watery solution of hydrochloride of anilin for a few seconds.

5. The sections are then decolorized in 75 per cent. alcohol till they are apparently free from stain; this will take from fifteen to thirty minutes.

6. They are then counter-stained with a solution of orange (one part of saturated watery solution of orange to twenty to forty parts of 50 per cent. alcohol), dehydrated, cleared, and mounted.

Where a positive diagnosis is important, a small piece of the tissue may be inserted under the skin of the thigh or abdomen of

¹ See *Med. Chronicle*, v. 1896, p. 17.

a guinea-pig. If tubercular, the animal will show signs of tuberculosis in two to three weeks (see below, Urine).

Cover-glass specimens of pure cultivations of the tubercle bacillus may be stained in warm carbol-fuchsin for two to five minutes, rinsed in the sulphuric or nitric acid solution, washed, dried, and mounted. They can also be stained by Gram's method, which usually brings out the beaded appearance very markedly. The distinction from the leprosy bacillus will be found at p. 812, from the smegma bacillus and other acid-fast organisms at p. 814.

III. *Urine*.—The tubercle bacillus is often very difficult to demonstrate in urine. The urine must be allowed to stand in a conical glass for twenty-four hours or centrifugalized, and cover-glass specimens prepared with the sediment and treated by one of the methods for sputum given above. Several specimens should be made and must be very carefully examined. It is important to exclude the smegma bacillus, and the urine is preferably drawn off by a catheter. Staining may be carried out by Housell's method, by which the smegma bacillus is decolorized, viz. after staining in warm carbol-fuchsin the specimen is washed and dried. It is then immersed in acid alcohol (3 per cent. hydrochloric) for ten minutes, washed in water, counter-stained for a few seconds in a saturated alcoholic solution of methylene blue, washed, dried, and mounted (see also p. 816).

If a diagnosis is of importance, inoculation should be resorted to. Two guinea-pigs are inoculated subcutaneously in the thigh or abdomen with 0.5 to 1 c.c. of the deposit from the sedimented or centrifugalized urine, or one may be inoculated subcutaneously, the other intraperitoneally. If tubercle bacilli are present the animals may show signs of tuberculosis as early as two to three weeks after inoculation. Delépine¹ recommends the inoculations to be made on the inner aspect of the leg about the level of the knee. The order of infection after inoculation is as follows: the popliteal, superficial and deep inguinal, and sublumbar glands, the retrohepatic, mediastinal and bronchial, deep cervical, and subscapular glands, the spleen, liver, and lungs. The inoculated

¹ *Brit. Med. Journ.* 1893, ii. p. 664. The results only apply to ordinary forms of tuberculosis, and not to certain modified forms such as lupus and the avian variety.

animals are killed in two to three weeks, dissected, and the lesions examined microscopically. Others inoculate two guinea-pigs, one subcutaneously in the abdomen, the other intraperitoneally. Negative results are nearly as valuable as positive ones.

In *feces*, it is not much use looking for tubercle bacilli unless definite yellow caseous particles can be found. These should be picked out, and films made and stained.

IV. *Milk*.—See section on Milk (Chapter XXI.).

V. *Agglutination Reaction*.—The method of agglutination has been proposed by Arloing and Courmont for the diagnosis of tuberculosis.¹ A special method has to be employed to obtain homogeneous cultures of the tubercle bacillus. A number of glycerinated potato cultures of the tubercle bacillus are prepared and incubated at 88°–89° C.; in a few, colonies develop having a fatty and shining appearance. These are picked out, sown in glycerin broth (6 per cent.), and the broth cultures are incubated at 88°–89° C., being well shaken several times every day; in a few the bacilli develop in an isolated manner, and these tubes are selected and kept for re-inoculating other glycerin broth tubes which are used in performing the test. The flat flasks (fig. 37) are better than tubes for obtaining satisfactory cultures. The cultures employed are preferably eight to twelve days old. For carrying out the reaction small sterile test-tubes are employed. For each test three dilutions of the serum are made, a 1 in 5, a 1 in 10, and a 1 in 20, and the tubes filled with these dilutions are allowed to stand in an inclined position (45°) for five to ten hours; the reaction may be observed both microscopically and macroscopically. In man the serum of normal individuals may agglutinate up to a dilution of 1 in 5, while in animals this is variable—imperceptible in the guinea-pig, rabbit, and calf; feeble in the goat; in the adult ox up to 1 in 5, but in the dog it may be up to 1 in 10 or even 1 in 20.

A positive serum reaction in a suspected subject is a sign of great value in establishing the diagnosis; a negative serum reaction is of less value. The technique, however, is difficult, and the test has not been much used for clinical purposes.

¹ *Zeitschr. f. Tub. u. Heilstättenwesen*, i. p. 11.

Koch¹ uses a dried culture of the tubercle bacillus, pulverizes it, and mixes a weighed quantity of the powder with 1000 times its weight of the following fluid: 0.85 per cent. salt solution containing 0.5 per cent. carbolic acid. A tenfold dilution of the serum is employed.

VI. *The Opsonic Method.*—The opsonic method has recently been much used. The general mode of carrying it out is that detailed at p. 192. But the tubercle bacilli used for phagocytosis should be suspended in 1.5 per cent. salt solution. Appropriate preparations of killed bacilli can be purchased; a particle the size of a large pin's head is taken and ground up in an agate mortar with a drop of the salt solution. The grinding is continued, with the addition of drops of salt solution from time to time, until a thin opalescent suspension has been prepared. This is allowed to sediment for half an hour, or is centrifugalized for 2-3 minutes, to deposit clumps. The films are best stained in an excess of carbol-fuchsin in the warm incubator for some hours, and then decolorized in 5 per cent. sulphuric acid or in acid alcohol; stronger acid tends to disintegrate the leucocytes.

VII. *The Tuberculin Reaction.*—The old tuberculin is used for diagnostic purposes; it is not perhaps very safe. A dose of 0.002 c.c. is injected subcutaneously, and the temperature taken four-hourly during the succeeding thirty-six hours. A rise of 2°-3° F. or more ensues a few hours after injection in tuberculous subjects. If no reaction occurs, another dose of 0.005 c.c. may be given after the lapse of some days.

This method has now almost completely been superseded by the cutaneous or ophthalmic reaction.

The Cutaneous Tuberculin Reaction.—Von Pirquet² discovered that when tuberculin is introduced into the superficial layers of the skin of tuberculous individuals, as in vaccination, a reaction occurs consisting of the formation of a papule with redness, slight swelling and exudation, and sometimes small vesicles. This reaction is usually at its height twenty-four to forty-eight hours after inoculation. In healthy individuals no reaction follows the inoculation. The method is to scarify a small spot on the forearm

¹ See *Lancet*, 1901, ii. p. 1704.

² *Wien. Med. Woch.* July 6, 1907.

through a drop of a dilution of the old tuberculin, and protect the patch with a simple dry dressing.

The Ophthalmo-Tuberculin Reaction.—Calmette has transferred the site of inoculation from the skin to the conjunctiva. He makes use of a dried preparation prepared by precipitating the old tuberculin with alcohol, of which a 1-100 solution is prepared in distilled water. One drop of this is instilled into the inner half of the conjunctiva of one eye. In tuberculous individuals a reaction follows, usually in six to sixteen hours after medication, consisting of a conjunctivitis, ranging in intensity from a local redness to a redness extending over the whole eye and having the appearance of an acute conjunctivitis. The reaction soon passes off without leaving ill effect. On the whole, the reaction appears to be fairly constant in tuberculous individuals, but absence of reaction is not certain proof that the case is not tuberculous.¹

VIII. *Tuberculin for Veterinary Use.*—In veterinary practice tuberculin is extensively used for the diagnosis of the disease in cattle. The dose of the various preparations in the market varies according to their strength; it corresponds to 0.1 c.c. or 0.2 c.c. of Koch's original tuberculin.

(a) The dose is injected subcutaneously in the neck.

(b) If possible the temperature of the animal should be taken morning and evening for two or three days previous to inoculation.

(c) The temperature should be taken immediately previous to inoculation.

(d) The temperature should be taken at the twentieth hour after injection, or, where it can be done, at frequent intervals from the twelfth to the twentieth hour.

(e) The reaction consists of a rise of temperature of 1.5° to 6° F. above the average normal, occurring eight to twelve hours after injection, and lasting twelve to fourteen hours, accompanied by some systemic disturbance.

(f) A healthy animal is unaffected by the injection, and if an animal be very extensively affected with tuberculosis the reaction may not be given, or may be masked by the febrile condition present.

¹ See articles in *Brit. Med. Journ.* and *Lancet*, 1907, ii., and 1908, i.

JOHNE'S DISEASE.

Johne's disease,¹ a bovine enteritis, is due to an acid-fast bacillus closely resembling the tubercle bacillus in morphology. It occurs in scrapings of the affected mucous membrane of the bowel, and also in sections of the intestinal wall. The Johne bacillus has not been cultivated, and is not inoculable into the guinea-pig or rabbit.

PSEUDO-TUBERCULOSIS.

The term 'Pseudo-Tuberculosis' (which is not a good one and should be discarded) has been applied to a number of different conditions which have as a common character the presence of tubercle-like nodules, but which are not caused by the tubercle bacillus. Such are produced by certain parasitic worms, by *Blastomycetes*, *Streptothrix* and *Aspergillus*, Protozoa, and by several bacteria.²

Pfeiffer's *Bacillus pseudo-tuberculosis* produces nodular deposits in the organs, accompanied by wasting, very like true tuberculosis. The disease, however, runs a more rapid course, death ensuing in the guinea-pig two to three weeks after inoculation. Guinea-pigs, rabbits, mice, and monkeys can be readily infected. The nodules consist of masses of round cells which undergo necrosis and caseation. The bacillus in the tissues is not readily stained, carbol-methylene blue being the best solution, as it is not acid-fast, nor does it stain by Gram's method. Morphologically it is a small rod 1-2 μ in length, usually non-motile, although it possesses a single flagellum or two flagella at one end.³ On gelatin it forms a whitish growth without liquefaction, like that of the colon bacillus but confined to the needle-track. It produces alkali, forms no gas,

¹ See McFadyean, *Journ. Comp. Path. and Therap.* xx., 1907, p. 48.

² See *Centr. f. Bakt.* xv., 1894, p. 501, and *Trans. Path. Soc. Lond.* 1. 1899, pp. 331-364. (Bibliog.)

³ Klein, *Rep. Med. Off. Loc. Gov. Board* for 1899-1900, p. 355.

and does not curdle milk. Broth remains clear, with a whitish stringy flocculent deposit. It grows readily and rapidly.

MacConkey has found that the fermentation reactions of this organism and of the plague bacillus are practically identical (see 'Plague'), and sterilised cultures of either will protect against the other.

Ovine caseous lymphadenitis, a disease of sheep simulating tuberculosis, is due to a short plump bacillus with rounded ends which stains well by Gram's method, and grows best on blood serum, on which it forms greyish colonies.¹

LEPROSY.

Leprosy, the Elephantiasis Græcorum or true Elephantiasis, is a disease which has existed and has been recognized from the earliest times among the Egyptians, Jews, Greeks, and Romans. It was undoubtedly somewhat prevalent in the British Isles from the twelfth to the fifteenth centuries, as the many leper houses and enactments against lepers testify. At the same time, no doubt a number of other skin diseases, syphilides, psoriasis, lupus, &c., were at that early period of medical diagnosis confounded with it. In the present day leprosy, although extinct in the British Isles, may be said to have a world-wide distribution, for it is met with in Iceland and Scandinavia, Russia and the Mediterranean coasts; in Persia, India, China, Siberia, and Japan; in Africa from north to south, in the American continent in many districts, and also in the Pacific Islands. Three varieties of leprosy are described—the tuberculated or nodular, the anæsthetic, and the mixed.

The mode of spread is probably by personal contact, possibly insects play some part. J. Hutchinson supposes that fish in the diet, particularly if stale, decomposed, or

¹ Sixteenth Ann. Rep. Bureau of Animal Indust. U.S.A. p. 638.

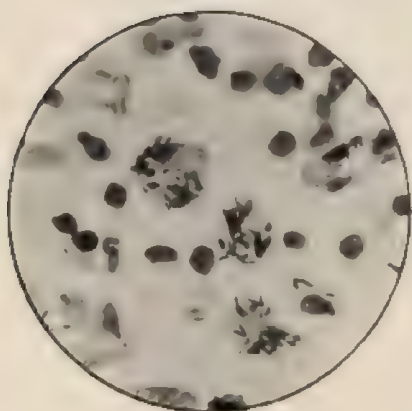
MANUAL OF BACTERIOLOGY

red, in some way is a causative factor, but he is alone in this view.

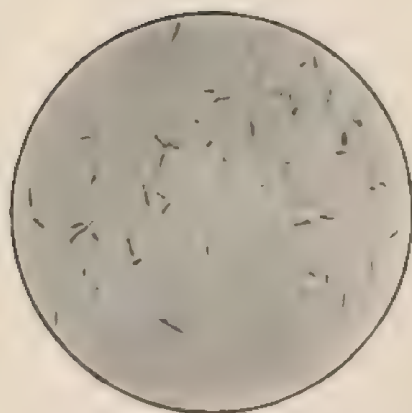
Throughout ancient and medieval times leprosy was held to be a contagious and communicable disease, as the stringent regulations in the Mosaic and other laws show. The segregation of lepers, and modern research bear this out, additional confirmation being given by the presence of a bacillus with very distinct lesions in the leprosy lesions.

Bacillus lepræ was discovered by Hansen in 1879. It resembles the tubercle bacillus, but is slightly longer; it probably does not form spores, though in preparations the same irregularity in staining—the occurrence of unstained intervals, the so-called 'beads'—is met with as in the tubercle bacillus, and is by some to be due to the presence of spores. The bacillus as obtained from the tissues is non-motile, stains with the ordinary anilin dyes, and by Gram's method, stains out the headed appearance very well and is

PLATE IX.



a. LEPROSY.
SECTION OF SKIN. $\times 1500$.



b. THE SMEGMA BACILLUS.
SMEAR PREPARATION OF SMEGMA. $\times 1500$.



seems to be usually the case.¹ Giant cells are occasionally present in the leprous nodules. One of the most constant and earliest situations in which the *B. lepra* is found is the nasal mucus.

Curiously enough, although the organism is present in such enormous numbers and is so readily demonstrable, there is considerable doubt whether it has been cultivated on artificial media or successfully inoculated into animals. Bordoni-Uffreduzzi isolated an organism from the bone-marrow of a leper and cultivated it on blood serum which possessed the same staining reaction as the *Bacillus lepra*, but differed from it slightly morphologically, being a little longer and thicker; and Campania described its cultivation anaerobically; but most attempts have failed.²

Van Houten³ claimed to have succeeded by cultivating in glycerin fish broth. The bacillus cultivated was acid-fast, and agglutinated with, and was sensitised by, lepers' serum.

Rost believed he had succeeded in cultivating the *B. lepra* in a salt-free medium, but subsequent investigation has not confirmed his results.

Deycke,⁴ by taking fragments of leprosy tissue and incubating for several weeks in physiological salt solution at 37° C. has obtained a growth of an acid-fast streptothrix, *S. leproides*. He is uncertain if this is a true growth of the leprosy bacillus. Injected into leprosy patients it seemed to produce a beneficial effect. The acid-fast property resides in a fatty substance which can be extracted with solvents, particularly benzoyl chloride. The fatty substance Deycke terms 'nastin'; it is a neutral fat, the

¹ See Bergengrün and Kanthack, *Trans. Path. Soc. Lond.* xlix., 1898, p. 336.

² See *Centr. f. Bakt.* xxv., 1899, p. 756.

³ *Journ. Path. and Bact.* viii., 1903, p. 260.

⁴ *Brit. Med. Journ.* 1908, i. p. 802.

glycerin ester of a fatty acid of high molecular weight. Injected into leprosy patients it sometimes produces marked reaction, sometimes not. In solution in benzoyl chloride it is much more active and Deycke hopes that it will act as a curative vaccine in leprosy.

A certain number of positive results of the inoculation of leprous material into the lower animals have been reported by Ortmann and others. Nicolle¹ has reported the successful inoculation of a macaque monkey, but most of the attempts have ended in failure; while the positive results are open to criticism and may be fallacious, for lepers not unfrequently suffer from coincident tuberculosis, and the animals therefore may have been infected with and have died from tuberculosis. The local lesion induced in animals may be simply inflammatory produced by the leprous material acting as a foreign body and the bacilli may be diffused without proliferating. Human beings have also been inoculated, but the positive results are all open to objection.

The differentiation of leprosy from tuberculosis, although the bacilli are so similar, does not in the majority of cases present much difficulty. The large number of bacilli present in the skin and in the leprous lesions elsewhere forms a marked distinction from tuberculosis, while the *Bacillus lepræ* stains more readily, and with watery solutions in a shorter time, than does the *Bacillus tuberculosis*, though this distinction is hardly marked enough for diagnostic purposes.

Cases of leprosy, both of the nodular and anæsthetic varieties, have been treated with injections of Koch's tuberculin, which has been found to produce a certain amount of reaction followed by some amelioration in their condition. Rost also used 'leprolin' with benefit (a boiled and concentrated extract of his supposed cultures).

¹ *Comp. Rend. Acad. Sc.* 1905.

Dean¹ and others have met with a leprosy-like disease in the rat. Nodules are found in the tissues which contain large numbers of an acid-fast bacillus closely resembling the *B. lepræ*. The organism could not be cultivated. Inoculated into rats it reproduced the disease after some months, but had no effect on guinea-pigs.

CLINICAL EXAMINATION.

1. If cutaneous nodules be present, one is clamped, pricked, and cover-glass specimens are prepared with the juice that exudes and stained as for tubercle. Large numbers of bacilli, having the same staining reaction as the tubercle bacillus and obtained from the cutaneous structures, are diagnostic of leprosy (the smegma bacillus may be present *on*, but not *in*, the skin).

2. In the tissues the diagnosis must be based on the presence of the bacilli in large numbers in the so-called leprosy cells.

Tissue sections are stained in the same manner as tuberculous material.

3. Leprosy is not inoculable in guinea-pigs.

N.B.—It must be remembered that lepers not unfrequently suffer from tuberculosis.

4. The differentiation of the leprosy from the tubercle bacillus by staining methods cannot be said to be satisfactory. By staining in a saturated aqueous solution of fuchsin in the cold for five to seven minutes, and subsequently decolorizing with acid alcohol (nitric acid 1 part, alcohol 10 parts), it is stated that the leprosy bacillus is stained, the tubercle bacillus not.²

THE SMEGMA BACILLUS.³

The smegma bacillus is an organism found in the smegma præputii, between the scrotum and thigh, and between the labia. It also occurs in the cerumen, occasionally on the skin, and possibly in the sputum.

¹ *Journ. of Hyg.* v., 1905, p. 90.

² See *Centr. f. Bakt.* xxv., 1899, p. 762.

³ See Czuplewski, *Münch. Med. Woch.* 1897, No. 43; Grünbaum, *Lancet*, 1897, i.; Neufeld, *Arch. f. Hygiene*, xxxix, p. 184; *Zeitschr. f. Hyg.* xxxix., 1901; and Moeller, *Centr. f. Bakt.* xxxi., 1902 (Originale), p. 278.

It is a small bacillus resembling the tubercle bacillus in size and appearance, and, like the latter, is difficult to stain, but when stained with carbol-fuchsin retains the colour after treatment with a 25 per cent. mineral acid (Plate IX., *b*) ; it is also Gram-positive. It has, therefore, to be distinguished from the tubercle bacillus in certain localities, viz. in urine and about the external genitals. It is non-inoculable on animals and does not usually grow in primary cultures on ordinary media, but can be isolated by the use of blood-, serum-, or nutrose-agar on which it forms delicate, ropy colonies. After isolation it grows freely on agar as a thin, slightly brownish, creamy layer, in which the bacilli may be very short but retain their acid-fast properties, on potato it forms minute (0.5–1 mm.) greyish colonies. It has been suggested that the syphilis bacillus of Lustgarten is identical with the smegma bacillus ; neither is decolorized by Lustgarten's permanganate method (*see* 'Syphilis'), but while the smegma bacillus after staining is with difficulty decolorized by acid, and is easily decolorized by alcohol, the reverse is the case with Lustgarten's bacillus.

STAINING AND DIFFERENTIATION.

Cover-glass specimens of smegma may be stained in exactly the same manner as for tubercle, after treating the preparations with ether to get rid of fatty material.

The urine should be drawn off with a catheter when it is to be examined for the tubercle bacillus ; this will generally exclude the smegma bacillus. Young and Churchman¹ conclude that the smegma bacillus is a scant invader of the male urethra, and that by washing the glans and irrigation of the urethra it may be eliminated from the urine.

If there is reason to suspect the presence of the smegma bacillus when staining for tubercle, Bunge and Tranteroth²

¹ *John Hopkins Hospital Rep.* xiii., 1906, p. 15.

² *Fortschrit. der Med.* xiv., 1896, Nos. 23 and 24. See also *ibid.* No. 9.

recommend that the cover-glass specimens should be treated as follows :

1. Immerse in absolute alcohol for three hours.
2. Immerse in 5 per cent. chromic acid for fifteen minutes.
3. Stain in warm carbol-fuchsin.
4. Decolorize in 25 per cent. sulphuric acid for two to three minutes.
5. Counter-stain in a concentrated alcoholic solution of methylene blue for five minutes.

The smegma bacillus will be decolorized by this method (see also p. 804).

Coles recommends (*Journal of State Medicine*, xii., 1904, p. 225) the following staining method :

1. Spread thin and even films on slides, and fix by heat in the ordinary way.
2. While still warm from the heat fixation flood with filtered carbol-fuchsin, and allow the preparation to remain for half a minute. Again warm for a few seconds over the flame without actual boiling. Allow it to stand and stain for seven minutes.
3. Wash thoroughly in running water, and then decolorize in either of the following solutions :

(a) *In Pappenheim's solution.*¹ Place the preparation in a wide-mouthed bottle containing the solution for not less than four and not longer than twelve hours. Wash, dry, and mount. Tubercle bacilli are the only organisms stained red.

(b) *In Pappenheim's solution without methylene blue.* Proceed as in (a) ; wash in water and counter-stain for a minute in weak aqueous methylene blue solution. The tubercle bacilli are brilliantly red.

(c) *In 25 per cent. sulphuric acid.* Pour on a few drops of the acid and allow it to act for half a minute. Pour off, and then place the preparation in a wide-mouthed bottle containing the

¹ Pappenheim's solution consists of 1 part of corallin (resolic acid) in 100 parts of absolute alcohol, to which methylene blue is added to saturation ; 20 parts of glycerin are then added.

acid for not less than sixteen hours and not more than twenty-four hours. Wash thoroughly, counter-stain with weak aqueous methylene blue. Tubercle bacilli are the only bacilli which retain the red.

Acid-fast Bacilli in Milk and Butter.—Numerous acid-fast bacilli have been obtained from milk and butter. They usually grow freely and quickly on agar and on gelatin without liquefaction, sometimes as a creamy layer, sometimes as a dry crinkled film, which may be pigmented (yellow, orange, pale brown, or brick red). Some are pathogenic to guinea-pigs by massive intra-peritoneal inoculation only, producing a plastic peritonitis, but not nodules in the organs. In culture, the bacilli are acid-fast, and occasionally resemble *B. tuberculosis*, but are generally thicker. (See Petri, *Arb. a. d. Kais. Gesundheitsamte*, xiv., 1897; Rabinowitsch, *Zeitschr. f. Hyg.* xxvi., 1897; Grassberger, *Münch. med. Woch.* 1899, Nos. 11 and 12; Tobler, *ib.* xxxvi.; Swithinbank and Newman, *Bacteriology of Milk* (Murray, 1903).

Grass Bacilli and Mist Bacillus.—Moeller isolated from a grass (*Phleum arvense*) an acid-fast bacillus which he termed the Timothy-grass bacillus; other grasses also yield acid-fast bacilli (Grass Bacillus II.). They grow readily on culture media, and are not so acid-fast as the tubercle bacillus. The Mist bacillus was isolated from dung, and is considered by Pettersson to be identical with the Timothy-grass bacillus. (See Moeller, *Deutsch. Med. Woch.* 1898, p. 376; Herr, *Zeitschr. f. Hyg.* xxxviii., 1901; Pettersson, *Berl. Klin. Woch.* 1899, p. 562.)

GLANDERS.¹

Glanders is a disease which has been known from the earliest times, being recognized by the Greek and Roman writers, by whom it was termed *μᾶλις* and *malleus* respectively. It is distinctly a disease of the horse, mule, and ass, but is also communicable to man and to certain other animals. It is caused by a small bacillus discovered by Löffler and Schütz in 1882.

¹ See McFadyean, *Journ. of State Med.* xiii., 1905, pp. 1, 65, and 125.

In the horse the lungs are always affected, and frequently the nasal mucous membrane (fig. 39). Nodules form which afterwards break down and ulcerate, and a muco-purulent discharge appears; in the older writings the name 'glanders' covered only these advanced cases of the disease. In 'farcy' the lymphatic vessels and glands

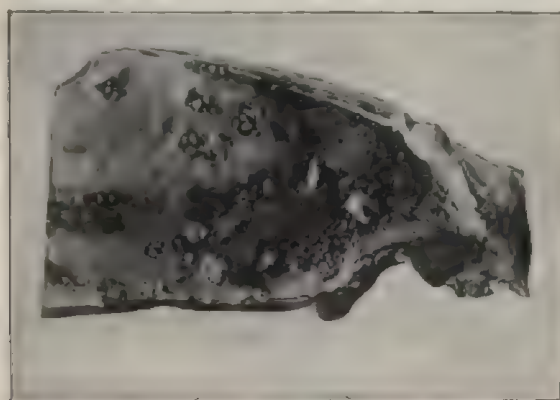


FIG. 39. NASAL SEPTUM OF GLANDERED HORSE, SHOWING
ULCERATION OF SCHNEIDERIAN MEMBRANE (McFadyen).

are affected, the enlarged glands being known as 'farcy buds' (fig. 40).

In man the disease is rare, an average of four deaths per annum being caused by it in this country. It occurs in two forms—the acute and the chronic. The former is a very serious affection accompanied by high fever, prostration, and delirium, and almost invariably fatal in from two to three weeks. The seat of infection is usually the hand or arm, the nasal mucous membrane being sometimes subsequently involved, and deposits may form in the lymphatic glands, internal organs, and muscles. In the chronic form intramuscular abscesses are frequent, from the breaking down of which indolent ulcers may result,

the disease runs a prolonged course of weeks or even months, and about half the cases end in recovery. In the early



FIG. 40.—HORSE AFFECTED WITH FARCY (McFadyen).

stage an eruption may develop on the forehead and face simulating very closely that of small-pox.

THE GLANDERS BACILLUS.

The glanders bacillus (*B. mallei*) is an obligatory parasite with the equine species for its normal host. It hardly grows on artificial media below about 20° C., and probably cannot maintain a saprophytic existence outside the animal body.

Morphology.—The glanders bacillus occurs in the tissues as a cylindrical rod with rounded ends, varying between 2 and 5 μ in length, and generally straight, though sometimes slightly curved. The bacilli are usually irregularly scattered, and do not tend to form colonies. In stained preparations they often appear more or less beaded, or may exhibit bipolar staining, but some stain uniformly. The bacilli from young cultures not more than twenty-four hours old are almost always short rods, a little thicker than

those found in the lesions (Plate X., a). In old broth cultures the surface growth is largely composed of filaments, which do not show any regular segmentation but may exhibit lateral branching, and may have club-shaped extremities. From these features some have inferred that the glanders organism is related to the *Actinomyces* group. The bacillus does not form spores and is probably non-motile, though in a hanging-drop cultivation made from a pure culture an extraordinarily active Brownian movement is observed.

Staining Reactions.—The bacillus is Gram-negative, and is not acid-fast, but from young cultures stains readily with the ordinary anilin dyes. In smears of glanders or farcy material, a simple staining with any of the basic anilin dyes, with subsequent decolorization with dilute acetic acid, suffices to demonstrate it if it is present in any number (which is frequently not the case), the only difficulty in recognizing the organism being the presence of deeply staining nuclear detritus. In sections, methylene-blue staining with decolorization in dilute acetic and mordanting with tannin gives the best results (p. 325). The bacillus shows dark staining dots when treated with osmic acid, suggesting fat globules (Shattock).

Cultural Characters.—The *Bacillus mallei* is an aerobic and facultatively anaerobic organism. It grows but slightly on gelatin at 22° C., what growth there is being pale brownish and without liquefaction. On glycerin agar it forms a thick cream- or slightly brown-coloured growth, and on blood serum a somewhat amber-coloured growth which afterwards becomes brownish. The growth on potato at 37° C. is most characteristic and practically diagnostic. If the surface of the potato is inoculated with a loopful of farcy pus or material from the centre of a glanders nodule, the resulting growth is usually not distinctly visible until the third day; when raised, trans-

lucent, viscid, amber-coloured colonies, like drops of honey, appear. With continued incubation the colonies coalesce, the growth becomes thicker and fawn-coloured, then reddish brown, and finally generally chocolate-brown. The growth is also odourless, limited to the site of implantation, and does not stain the potato. Broth or glycerin broth becomes uniformly turbid, and after a week or so patches of a whitish surface scum form, and after three weeks the broth is nearly covered with this surface growth, which is slimy and easily broken up on shaking. Broth cultures give the indole reaction. Litmus glucose agar becomes pink. Milk is not coagulated.

Resistance to Germicides &c.—The glanders bacillus is but little resistant, and cultures frequently die out in a month or so. Complete desiccation of nasal discharge, farcy pus, or bacilli from cultures at 37° C. is frequently fatal in twenty-four to forty-eight hours. Young broth cultures are soon destroyed by bright sunlight, and an exposure of ten minutes to a temperature of 55° C. is fatal to the cultivated bacilli. A 3 per cent. solution of carbolic acid, a 1 per cent. solution of potassium permanganate, and a 1 in 5000 solution of corrosive sublimate are fatal in 2-5 minutes.

Pathogenicity &c.—The glanders bacillus varies considerably in virulence, and under continued cultivation may become almost non-pathogenic.

Glanders is met with exclusively among horses, asses, and mules, and man is infected from these animals, nearly all cases of human glanders being among ostlers, grooms, and coachmen, and the usual mode of infection is by farcy pus or nasal discharge coming in contact with a cutaneous wound or abrasion. A remarkable immunity, however, is enjoyed by the slaughterers who have to deal with the carcasses of glandered animals and who might be supposed to run the greatest risk. But it must be remembered that

Babes frequently found at the post mortem on persons who had to do with horses, and who died from diseases other than glanders, encapsuled glanders nodules in the lungs and internal organs, suggesting that the disease may often be latent in man, who would appear to be relatively insusceptible, and that infection may be possible by inhalation. In the horse glanders is readily transmissible experimentally both by ingestion and by inoculation, and ingestion is probably the common mode of infection naturally, infection by inhalation occasionally occurring. Even when glanders bacilli are administered experimentally by the mouth in the horse, the lesions may be most prominent in, or even be confined to, the lungs. In the horse, the disease has periods of epidemic prevalence, and is particularly frequent in London. In 1892 there were 3000 equine cases in Great Britain, in 1903 there were 2499 cases, and nearly 90 per cent. of all cases occur in the Metropolitan area. These, it is to be noted, were cases in which the disease was well developed and manifest, but there are also numerous others in which it is latent. Guinea-pigs and field-mice are highly susceptible to the disease, which may also be contracted by some of the Carnivora, such as the cat, lion, and tiger, by inoculation or by feeding on diseased carcasses. The rabbit, sheep, and dog are but slightly susceptible, while cattle, swine, and house-mice are stated to be immune. Shattock¹ found that the white mouse is somewhat susceptible, and suggests that in all probability the house-mouse will be found to be similarly so.

In the horse, the most constant seat of glanders lesions is the lung, and McFadyean states that no case of glanders with lesions elsewhere than in the lungs, and with these organs unaffected, has ever been recorded. In nearly every case of farcy, also, nodules are present in the lungs. The lung lesions have the form of rounded, firm, or shotty

¹ *Trans. Path. Soc. Lond.* lix., 1898, p. 333.

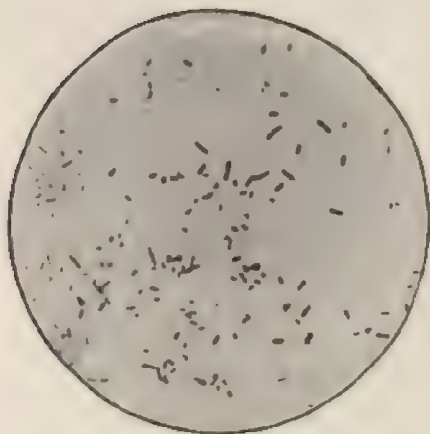
nodules. The number present is variable, rarely less than a dozen, exceptionally there are hundreds, fairly evenly distributed throughout the lung tissue. The nodule commences as a collection of polymorphonuclear leucocytes, around which a zone of congestion is present. Later, the alveolar walls undergo necrosis, and the leucocytes necrose and disintegrate, but their chromatin persists as rounded fragments which retain their affinity for nuclear stains (chromatotaxis). The nodule may become surrounded with a layer of thin fibrous tissue, between which and the necrotic central area a zone of endothelioid cells with giant cells may be present (Plate X., *b*).

The lesions of farcy are at the onset histologically identical with the glanders nodule, but by the progressive liquefaction of the tissues actual abscesses form.

The lesions set up in an inoculated guinea-pig are very characteristic and can be used for diagnostic purposes. With a very virulent culture, such as can be obtained by several passages through a susceptible animal, a guinea-pig may die in four or five days, and the post-mortem lesions are slight, consisting of some caseation at the seat of inoculation and slightly enlarged spleen, which contains a few small yellowish nodules resembling miliary tubercles. The material from human cases as a rule seems more virulent than that from the horse, and death of the guinea-pig often ensues a few days after inoculation.

Usually the culture or material from a glandered horse does not produce death of a guinea-pig until a lapse of two or three weeks. A male guinea-pig being chosen, the changes observed are caseation followed by ulceration at the seat of inoculation, when this is done subcutaneously, and great enlargement of the testicles; on cutting into these they are found to be partially or almost entirely converted into a pasty caseous material, while the skin covering them is so adherent that it can only be detached by cutting, and the

PLATE X.



a. THE GLANDERS BACILLUS.
COVER-GLASS PREPARATION OF A PURE CULTURE. $\times 1000$.



b. SECTION OF A GLANDERS NODULE, SHOWING GIANT CELLS
(AFTER McFADYEAN).



spleen is very much enlarged and studded with small yellowish nodules. In a female guinea-pig the ovaries are attacked. These appearances are of importance in the diagnosis of the disease. The difficulty of finding the bacillus in the discharges by microscopical and staining methods is so great that these cannot be employed with any certainty. Löffler and Straus therefore recommend the inoculation of a male guinea-pig intraperitoneally with the discharge or other material. If the glanders bacillus is present the lesions just described rapidly ensue, and the diagnosis is established in four or five days (Straus's test¹). At the present time the inoculation method has been almost entirely superseded by the introduction of mallein, the former being reserved for clinical diagnosis in man.

McFadyean found that the blood of a glandered animal produces agglutination or clumping of the glanders bacillus similar to that obtained in the agglutination (Widal) test for typhoid, and has suggested this reaction as a means of diagnosis. As an aid to the clinical diagnosis of the disease in man it is doubtful if the method of serum diagnosis can be applied, for Foulerton² found that typhoid and diphtheria sera also produce agglutination of the glanders bacillus.

Torins.—Mallein, a preparation analogous to tuberculin, is prepared by growing a virulent glanders bacillus for a month or six weeks in glycerin veal-broth in flat flasks such as are employed for tuberculin (fig. 37), so that there is free access of oxygen. The culture is then autoclaved for fifteen minutes at 115° C., filtered through a Berkefeld filter, concentrated to one-fourth of its volume, and mixed with an equal volume of a $\frac{1}{2}$ per cent. solution of carbolic acid. This yields an active mallein, 1 c.c. of which is a dose, and gives a good reaction. Like tuberculin, it has

¹ See also Nicolle, *Ann. de l'Inst. Pasteur*, xx., 1906, Aug., Sept., and Oct.

² *Lancet*, 1897, i. p. 1201.

little or no curative properties, though a few cases of cure by prolonged use have been reported by Babes and others, but is used for diagnostic purposes; and veterinary authorities are unanimously agreed that it is one of the most certain means we possess for diagnosing glanders in the horse. Injected into an unglandered horse little or no effect is produced, but in a glandered animal, about twelve hours after injection, the temperature rises 1.5° to 3° C. above the normal, a large and painful swelling forms at the seat of inoculation (it may be as large or even larger than half a cocoanut), while any affected lymphatic vessels or farcy buds become swollen. Reaction may, however, be produced in the absence of glanders if the horse is being treated with bacterial products, toxins, &c.¹

The greatest care should be exercised when working with glanders material or cultures, several fatal laboratory accidents having unfortunately happened to investigators.

CLINICAL EXAMINATION.

1. Prepare and stain film preparations of the pus or discharge in Löffler's blue, with subsequent partial decolorization in 4 per cent. acetic. The ordinary pyogenic cocci will not be found unless a secondary infection has occurred, and the material may appear sterile, for the glanders bacilli may be very scanty.

2. Several tubes of glycerin-agar and potato should be inoculated and incubated at 37° C. for seventy-two hours. On the agar, colonies of the glanders bacillus will develop in twenty-four to thirty-six hours, but the potato will not show the characteristic amber growth under forty-eight to seventy-two hours.

3. It will usually be necessary (in man, at any rate) to confirm the diagnosis by an inoculation experiment. A fully developed male guinea-pig is chosen and a little of the discharge, or an emulsion of the material (0.5 to 1 c.c.), is injected intraperitoneally, if the material be fairly sterile, but if not, subcutaneously. In

¹ See Sudmersen and Glenn, *Journ. of Hygiene*, viii., 1908, p. 14.

three to five days the animal should show the characteristic swelling of the testicles if the material be glandered.

4. In animals the mallein test may be applied :

(a) The dose is injected subcutaneously in the neck over the vertebrae, about midway between the jaw and the shoulder, the syringe and skin having previously been disinfected.

(b) If possible the temperature of the animal should be taken morning and evening for two or three days previous to inoculation.

(c) The temperature of the animal should be taken at the twentieth hour after inoculation, or, if possible, at frequent intervals from the twelfth to the twentieth hour.

(d) A complete reaction comprises (1) a rise of temperature of more than 2.5° F., (2) an extensive hot and painful swelling at the seat of inoculation.

(e) Systemic disturbance, such as prostration, loss of appetite, shivering, &c., may occur.

(f) The temperature reaction is unreliable in all cases in which the temperature at the time of inoculation is 2.5° F. above the normal. In such cases, if there be any suspicious clinical signs to assist, reliance may be placed upon the local swelling.

5. In animals the agglutination reaction may be applied, i.e. testing the effect of a 1 : 10 or 1 : 20 solution of the blood serum on the glanders bacillus (see p. 172). Moore and Taylor¹ consider that the diagnosis of glanders in horses by the agglutination method is easier and quite as accurate as by mallein. In man this test might give an inconclusive result (see *ante*).

6. In the tissues the glanders bacillus is difficult to demonstrate. Sections may be stained for half an hour with carbol-methylene blue, treated with 4 per cent. acetic for a few seconds, washed, and rapidly dehydrated with alcohol, cleared and mounted. McFadyean recommends, after treating with the acetic and washing, flooding with a saturated solution of tannic acid in water for fifteen minutes, washing, counter-staining in a 1 per cent. aqueous solution of acid fuchsin for fifteen to thirty seconds, washing, dehydrating, and clearing in cedar oil.

Twort's method may also be employed (see section on *Amoeba coli*, 'Clinical Diagnosis').

¹ *Journ. of Infect. Diseases*, Sup. No. 3, May 1907, p. 85.

CHAPTER X.

TYPHOID FEVER—PARA-TYPHOID FEVER—BACILLUS ENTERITIDIS
AND THE GÄRTNER GROUP—SWINE FEVER—BACILLUS DYSEN-
TERIÆ—BACILLUS COLI.

The organisms considered in this chapter form a natural group or family, and pass as it were by gradations in cultural characters from the typhoid bacillus to the colon bacillus. Löffler classes them together in a family, the Typhaceæ, which, according to the reactions of the organisms included in it on certain culture media, is divided into the sub-families. These culture media are: (1) the *typhoid solution*, an aqueous solution containing 2 per cent. peptone, 1 per cent. nutrose, 1 per cent. grape sugar, 5 per cent. lactose, and 1.5 per cent. normal potash; (2) the *para-typhoid solution*, having the same composition with the exception that the glucose is omitted. To 100 c.c. of the solution in each case 1 c.c. of a 0.2 per cent. solution of chemically pure malachite-green crystals (Hoechst, No. 120) is added.

Löffler's classification is as follows:

Family.—Typhaceæ.

Sub-family 1.—Typhææ. This group does not ferment the typhoid solution, but leaves it unchanged or may precipitate it. The para-typhoid solution is not fermented, is unchanged or may become milky. The following organisms are contained in it: (a) *B. typhosus*, actively motile, precipitates the typhoid solution, has no action on the para-typhoid solution. (b) *B. dysenteriae* (Flexner), motile,¹ does not precipitate the typhoid solution, and the para-typhoid solution is unchanged. (c) *B. dysenteriae* (Shiga-Kruse), non-motile, has no action on either solution, but if the green be omitted from the typhoid solution, it is precipitated. (d) *B. typhosimilis*, motile, does not precipitate the typhoid solution,

¹ It is usually stated to be non-motile.

but renders the para-typhoid solution milky; occurs in impure water and fæces. (e) *B. pseudodysenteriae* does not alter either solution, is not agglutinated by either Flexner or Shiga-Kruse serum.

*Sub-Family 2.—Josarcem.*¹ This group ferments the typhoid solution with gas-formation and frothing, and alters the para-typhoid solution. It includes the following organisms: (a) *B. paratyphosus* A, motile, ferments the typhoid solution, renders the para-typhoid solution a darker green; pathogenic for mice *per os*. (b) *B. paratyphosus* B, motile, ferments the typhoid solution, decolorizes the para-typhoid solution; occurs in man, ox, sheep, swine, horse. (c) *B. typhimurium*, resembles (b); pathogenic *per os* only for mice and field-mice. (d) *B. Danysz*, resembles (b) and agglutinated by *paratyphosus* B serum; pathogenic for rats and mice. (e) *B. psittacosis*, resembles (b); pathogenic for parrots and man. (f) *B. enteritidis-isosarcinus* (Gärtner), resembles (b), but not agglutinated by *paratyphosus* B serum; sometimes forms thermostable poisons. (g) *B. isosarcinus*, n. sp., from cases of food-poisoning; resembles (b), not agglutinated by Gärtner or *paratyphosus* B serum. (h) *B. suispestifer*, resembles (b); pathogenic.

Sub-Family 3.—Colæ. This group ferments both solutions with gas-formation and frothing. Includes (a) *B. coli*, slightly motile or non-motile; ferments both solutions. (b) *B. paracoli*.

In addition, all the organisms are agglutinated by their homologous sera.

TYPHOID FEVER.²

The specific organism of typhoid fever is a bacillus originally isolated by Eberth in 1880, and more closely studied by Gaffky in 1884.

The Eberth-Gaffky bacillus, or *Bacillus typhosus*, is best observed in sections of the spleen, in which it occurs in groups or colonies consisting of short rods with rounded ends, each measuring about 3 μ in length. It has also been demonstrated in the mesenteric glands and liver, in the

¹ From *læs*, poison, and *σάρκς*, flesh.

² See Horton Smith, *Lancet*, 1900, i. p. 821 *et seq.*

swollen Peyer's patches before ulceration, and in other situations.

In order to obtain pure cultivations it is preferable to make use of the spleen. The organ is washed and then cauterized lineally by means of a red-hot iron in order to destroy the saprophytic bacteria on and near the surface. An incision is made with a sterilized knife through this cauterized area, and a little of the splenic pulp is taken with a sterilized platinum needle and inoculated on to tubes or plates, preferably of litmus lactose, Conradi-Drigalski, or malachite-green agar. These are incubated at 37° C. for twenty-four to forty-eight hours, and the growths which develop are examined and tested by microscopical and culture methods. The organism may also be obtained by cultivation from the blood of a patient (p. 345). The following are the characters of the *Bacillus typhosus*.

Morphology.—Bacilli with rounded ends 2 to 3 μ in length and 0.6 μ broad. It is, however, in cultivation a markedly pleomorphic organism, and very short rods, long rods, and thick filaments 10 to 30 μ in length occur; the latter are known as involution forms (Plate XI., a). It does not form spores, but granulation and vacuolation may be observed in the protoplasm.

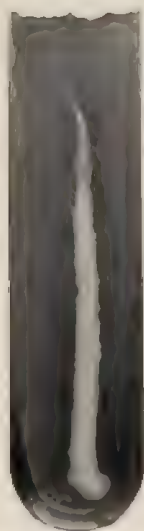
It is actively motile and possesses a number of flagella, arranged peritrichically both at the poles and sides (Plate XI., c). The flagella are long and wavy and average 8 to 12 in number, which is an important point of differentiation from the *Bacillus coli*, which usually has only 3 or 4. It stains by the ordinary anilin dyes, but not by Gram's method. In stained preparations, unstained spaces or vacuoles may frequently be seen.

Cultural Characters.—The *B. typhosus* is aerobic and facultatively anaerobic and grows well on the ordinary culture media. On agar it forms a thick, moist, greyish layer. On gelatin it grows slowly, and the growth, which

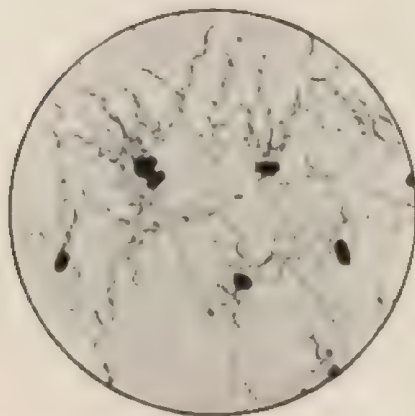
PLATE XI



a. *BACILLUS TYPHOSUS*.
COVER-GLASS PREPARATION OF A PURE
CULTURE. $\times 1500$.



b. GELATINE CULTURE OF
B. TYPHOSUS, 6 DAYS OLD.



c. *BACILLUS TYPHOSUS*.
COVER GLASS PREPARATION SHOWING FLAGELLA. $\times 1500$.



is usually scanty and confined to the needle-track, is white and shining and somewhat irregular (Plate XI., b). The colonies in gelatin are visible in about forty-eight hours, and form small roundish white points, which are granular and brownish in colour by transmitted light. In broth it produces a general turbidity, without film formation. The growth on potato acid in reaction is somewhat characteristic; it forms a moist, grey, shining layer which is almost invisible. If, however, the reaction of the potato is neutral or alkaline, the growth may be yellowish. The *B. typhosus* grows well in milk with slight permanent acidity, but without coagulation.

Acid is formed in small quantity during its growth in many media (volatile fatty acids, and lactic acid), which can be demonstrated by cultivating in litmus milk, or in litmus glucose media, and the organism will grow in slightly acid media. Neither gas nor indole¹ is formed in cultures; acid is produced from glucose but no gas, lactose is unacted upon. The fermentation reactions on various media are given in the table on p. 355, and are there contrasted with those of the *B. coli* and other organisms (see also p. 359). Chatterjee² finds that agar on which the typhoid bacillus has been grown contains toxic substances, and that if the agar be scraped so as to remove all growth, the typhoid bacillus will not again grow on this agar, but other bacilli will do so, so that this forms a test for the typhoid bacillus.

Pathogenicity.—In cases of typhoid fever in man the *Bacillus typhosus* is widely distributed in the body, in the various tissues, and in the blood, from which it may be obtained by cultivations made from at least 0·5 c.c. (see 'Clinical Diagnosis,' p. 345). The bacillus is constantly

¹ Occasionally a feeble indole reaction may be obtained by careful testing.

² *Trans. XIV. Internat. Cong. of Hygiene* (Berlin, 1907), Bd. iv. p. 34.

present in the blood from the commencement of the disease, though not in large numbers, and cultures from the blood in competent hands result in the recovery of the organism in approximately 100 per cent. of the cases; in the later stages of the disease it is less frequently recovered.¹ In addition to being present in the Peyer's patches, mesenteric glands, and spleen, the *B. typhosus* has been found in the rose-spots of the eruption, in the sweat, in the sputum and lungs in the pulmonary complications, and in the urine. In the urine it is so frequently present that special disinfection should be practised, more particularly during convalescence, and in some cases it may be so abundant as to produce a turbidity (typhoid bacilluria) and cystitis. It is also pyogenic, and occurs (usually in pure culture) in concurrent or post-typhoidal complications—e.g. empyema, abscesses, osteomyelitis, suppurating ovarian cysts, &c. Clumps of bacilli in the gall-bladder have been suggested as the nuclei of gall-stones, and the bacilli may be so numerous in the gall-bladder and bile ducts as to cause cholecystitis and cholangitis. The organism is by no means easy to isolate from the stools, simple plate cultivations usually fail, and the best medium to employ is the Conradi-Drigalski or malachite-green agar (see 'Water').

Injected intraperitoneally into mice and guinea-pigs the *B. typhosus* usually produces death, and the same result follows from intravenous injections in rabbits, but by continuous cultivation it loses its pathogenic properties. Given by the mouth, no result follows, and the same is the experience of most observers who have fed animals on typhoid stools; a disease process analogous to typhoid fever in man has rarely been induced experimentally. Remlinger² states that by feeding young rabbits on vegetables, cabbage, &c. soaked in water to which had been

¹ Coleman and Buxton, *Amer. Journ. Med. Sci.* June 1907.

² *Ann. de l'Inst. Pasteur*, xi., 1897, p. 829.

added some culture of the typhoid bacillus he has succeeded in inducing a condition resembling typhoid fever in man. The charts which accompany the paper show a typical rise of temperature, a period of pyrexia with morning remission, followed by a typical fall of temperature. The animals suffered from diarrhoea, and their blood gave the agglutination reaction. Post mortem, the intestine was congested and filled with yellow diarrhetic matter, the Peyer's patches were swollen and in some places commencing to ulcerate. The spleen was increased to two or three times its normal size, and cultures of the typhoid bacillus were obtained from it. The morbid effects following intraperitoneal inoculation are similar to those produced by the *Bacillus coli* and other organisms.

The proof of the causal relation of the *Bacillus typhosus* to enteric fever is based on the following facts. It is met with in the tissues in cases of enteric fever, can be obtained from the spleen during life by puncturing with a hollow needle, and may be isolated from the urine and blood during the course of the disease, and is not met with in other diseases. The writer has had under his care three cases, and knows of several others, in which the disease was almost certainly contracted in the laboratory from working with pure cultures. The blood and blood serum of an animal immunized against the *B. typhosus* are found to bring about cessation of movement and agglutination or aggregation of the bacilli in a broth culture of the organism. A similar result occurs when the serum of a patient, in the second week of an attack of typhoid fever, acts on the *B. typhosus*, the reaction not occurring with healthy individuals or in other diseases (Plate XII., a). This indicates that in the body of an individual suffering from typhoid fever the same substances are formed as in an animal artificially immunized by cultures of the *B. typhosus*. This reaction is now recognized as a valuable clinical test

in doubtful cases of enteric fever (the 'Widal' or agglutination reaction¹).

The Agglutination Reaction.—For the method of carrying out the agglutination reaction see p. 345. Various precautions have to be taken to ensure a reliable result. Cultures of the typhoid bacillus may contain pre-formed clumps of bacilli, these may be removed by filtering the culture through a moistened filter-paper; some strains agglutinate better than others; *normal* serum will generally agglutinate in a dilution up to 1 in 3 or 4, but occasionally is much more active. Dead bacilli may be used. The reaction is not obtained before the sixth or seventh day of fever, occasionally not until much later. Very rarely the reaction seems to be intermittent. The blood may retain its agglutinating power for years after an attack, and inoculation with Wright's vaccine also confers agglutinative properties. Cases do occur in which agglutination is absent throughout, but they are rare and often tend to be severe and to terminate fatally. If the blood during the course of an attack fails to give a reaction when tested on three occasions at intervals of three to four days, it is improbable that the case is one of typhoid fever. Moreover, cases occur, simulating typhoid closely, due to infection with the so-called para-colon or para-typhoid bacilli. These 'para' bacilli belong properly to the *B. enteritidis* group of organisms (see p. 348). If a positive reaction be obtained, yet the case does not seem to be one of typhoid, a previous attack or inoculation with Wright's vaccine must be excluded. The previous injection of a typhoid anti-serum into the patient might induce a non-typhoid infection to give the reaction.

Gwyn² found that out of 265 cases diagnosed as typhoid and accurately studied, only one persistently failed to give

¹ Some controversy has arisen as to the discoverer of this reaction. Grünbaum claims to have first observed it.

² *Johns Hopkins Hosp. Bull.* viii. 1900, p. 387.

the reaction. The blood of this case, however, reacted typically with a Gärtner-like organism obtained from the blood (a case, therefore, of para-typhoid infection with all the symptoms of typhoid fever).

Johnson and McTaggart¹ state that typhoid blood dried for sixty days still gives a typical agglutination reaction. An incomplete reaction was occasionally obtained as early as the end of the second day, and the complete reaction was rarely delayed beyond the fifth day. They also noticed that the blood of the horse often produced clumping &c. of typhoid bacilli, indistinguishable from an agglutination reaction with typhoid blood; but the same agglutinating effect was also produced on the colon bacillus. Many chemical substances also produce agglutination of typhoid bacilli, so that it is necessary to exclude them in making a diagnosis. For example, corrosive sublimate (0·7 : 1000), alcohol, salicylic acid, vesuvin, and safranin (1 : 1000) agglutinate, while carbolic and lactic acids, chloroform, caustic soda, and ammonia do not; the last two only, provided the test typhoid emulsion be made with distilled water. Safranin has a powerful agglutinating action on the typhoid bacillus, but not on the colon bacillus.²

There is no constant connexion between the activity of agglutination and the severity of the disease, nor does the bactericidal action of the blood serum undergo any constant alteration as a result of the typhoid infection. The agglutinating and bactericidal actions of the blood serum are not due to the same substance, for while the bactericidal action is destroyed by heating to 55° C. for half an hour, the agglutinating action is unaltered by this treatment.

Torins.—From cultures of the typhoid bacillus Brieger

¹ *Brit. Med. Journ.* 1896, ii. p. 629.

² Malvoz, *Ann. de l'Inst. Pasteur*, xi., 1897, p. 582.

isolated a base which he termed typhotoxin and which is isomeric with gudinine. In animals it produced salivation, profuse diarrhoea, paralysis, and death. Brieger and Fränkel have also isolated from cultures a toxic protein body. Fenwick and Bokenham¹ extracted from spleens of typhoid fever patients a proteose, an alkaloid, and a fatty residue. The proteose produced fever, anorexia, and loss of weight in guinea-pigs and rabbits, but the alkaloid and fatty matter were without effect.

The toxins of the typhoid bacillus, however, seem to be largely intracellular, and filtered broth cultures are usually almost non-toxic. Sidney Martin² by cultivating in a protein medium was able sometimes to obtain a toxic filtrate, a few c.c. of which produced lowered temperature, diarrhoea, and death. Macfadyen and Rowland³ by disintegrating large quantities of typhoid bacilli, filtering, and so obtaining the intracellular constituents in the filtrate, found that small doses of the latter produced a transient rise of temperature in guinea-pigs and a loss of weight which was soon recovered from. Animals so treated were protected against a certain lethal dose of typhoid bacilli, and their blood exhibited agglutinative and bacteriolytic properties towards the typhoid bacillus. Macfadyen⁴ later obtained the intracellular juices of typhoid bacilli by disintegration after freezing with liquid air, and found it to be very toxic to guinea-pigs by intraperitoneal, and to rabbits by intravenous, inoculation. Cultures of the *Bacillus typhosus* do not give the 'diazo' reaction.⁵

Survival of the Typhoid Bacillus in the Body.—Bacilli may persist in the spleen for weeks, in the gall-bladder for

¹ *Brit. Med. Journ.* 1895, i. p. 801.

² *Ibid.* 1898, ii. pp. 11 and 73.

³ *Centr. f. Bakt.* xxx. p. 753.

⁴ *Proc. Roy. Soc. Lond. B.* lxxi., 1902, p. 77.

⁵ Hewlett, *Brit. Med. Journ.* 1896, i. p. 136.

years, and in suppurative lesions for six years or more. Foster and Kayser obtained pure cultures from the gall-bladders of seven out of eight cases, and in 2 per cent. of the cases this 'cholecystitis typhosa' becomes a chronic process and typhoid bacilli may be discharged into the bowel for long periods. Dean¹ found this to be the case in a patient who had had enteric fever twenty-nine years previously. Such 'typhoid carriers' have been the subject of much investigation recently. A. and J. Ledingham² record three instances met with in an asylum in which mysterious cases of typhoid had occurred—31 cases during fourteen years. Davies and Walker Hall³ relate similar outbreaks, the carrier in this case being a woman who had suffered from enteric fever in 1901, milk serving as the vehicle of transmission, and a number of other instances have been recorded. Three-fourths of the cases are women (and three-fourths of the cases of gall-stones occur in women), and usually the serum of the carriers gives a marked agglutination reaction, and their stools frequently contain such large numbers of typhoid bacilli that these largely replace the natural bacterial flora of the intestine and may often be recovered from the stools by simple plating. Obviously the typhoid carrier is a source of serious risk to the community, and mysterious outbreaks of enteric fever, ascribed by some in the past to a 'de novo' origin of the specific organism, become explicable. The typhoid bacillus may occur in the contents of ovarian cysts, usually causing suppuration, and may survive for months—twelve in a case recorded by Taylor⁴—after the attack of typhoid.

Survival of the Typhoid Bacillus outside the Body.—The *Bacillus typhosus* has been isolated in a few instances

¹ *Brit. Med. Journ.* 1908, i. p. 562.

² *Ibid.* p. 15.

³ *Proc. Roy. Soc. Med.* i. 1908, Epidemiolog. Sect. p. 175.

⁴ *Journ. Obstet. and Gynecol. Brit. Empire*, Nov. 1907.

from WATER SUPPLIES which have become infected, and have given rise to epidemics, as in the case of the Worthing epidemic in 1892. This is the exception, however, and the isolation of the typhoid bacillus from an infected water is a very difficult matter on account of the fact that the bacillus may have died out before the investigation is commenced, that it is generally in a small minority and admixed with numbers of coliform organisms, and that until recently no medium was available which inhibited the growth of the coliform organisms without at the same time inhibiting the growth of the *B. typhosus*. By the use of malachite-green media the last-named difficulty seems to have been overcome (see 'Water').

In sterilized waters, including distilled water, the *Bacillus typhosus* maintains its vitality for upwards of a month, and in some cases for much longer. The survival is not necessarily longer in an organically polluted water than in a pure water. Infecting sterilized Thames water (from the Temple Embankment) and sterilized tap-water of the Chelsea Waterworks with typhoid cultures, the writer found that, examining small quantities (1 c.c.) of the water, the bacillus appeared to die out in the former in two to three weeks, in the latter in four to five weeks.

The survival of the typhoid bacillus in natural waters must be influenced by many circumstances—temperature, chemical composition, struggle for existence with the natural bacterial flora, &c., of the water. Experiments by Russell & Fuller,¹ in which the organism, suspended in collodion sacs, was subjected to the action of lake water, indicated that the maximum was eight to ten days. Houston² does not altogether accept these results, and in some carefully carried out experiments with raw Thames, Lee, and New River waters artificially infected with varying

¹ *Journ. Infect. Diseases*, Sup. No. 2, Feb. 1902, p. 40.

² *First Rep. on Research Work*, Metropolitan Water Board, 1908.

numbers of typhoid bacilli, in which 100 c.c. of the water were examined, found that in none of eighteen experiments was a negative result obtained in four weeks, and it was only after nine weeks that the typhoid bacillus could not be isolated from this quantity in all the experiments. In aerated (CO_2) waters the *B. typhosus* does not survive a fortnight.

The methods of isolation from water are given in Chapter XXI.

The *Bacillus typhosus* may gain access to shell-fish, oysters, mussels, cockles, &c., particularly if obtained from sewage-polluted laying, as has been proved by Nash, Collingridge, Klein, and others. Such polluted shell-fish may give rise to typhoid epidemics—as at Winchester and Southampton in the case of oysters, and in the case of cockles derived from the Thames Estuary and imperfectly cooked to typhoid cases. From many samples of oysters subjected to contamination Klein obtained the colon bacillus in large numbers, and from one oyster a cultivation in phenol broth gave a pure culture of the typhoid bacillus. Further, by inoculating oysters, kept in tanks of sea-water, with typhoid culture typhoid bacilli were obtained from their interior four, nine, sixteen, and even eighteen days from the commencement of the experiment, the oysters showing no abnormal condition, while the typhoid bacilli recovered from them and from the tank water retained unimpaired all the characters of a typical typhoid bacillus.¹ (See also Chapter XXI.)

As regards the vitality of the *Bacillus typhosus* in sewage, Parry Laws and Andrewes² showed, in the first

¹ On pathogenic organisms in shell-fish see 'On Oyster Culture in Relation to Disease' (Loc. Gov. Board Rep. 1896); Rep. Med. Off. Loc. Gov. Board for 1899-1900, p. 574; Houston, Fourth Report of the Sewage Commission, vol. iii., 1904; and McWeeney on Shellfish Layings, Rep. Loc. Gov. Board, Ireland, 1904.

² Reports to the London County Council, No. 216, 1894.

place, that in ordinary sewage the mathematical chances of detecting the bacillus are extremely remote, but that in the sewer from a typhoid block at the Eastern Hospital, Homerton, where the stools had not been disinfected for two days, a bacillus was isolated which was identical with the *B. typhosus*. In sterilized sewage inoculated with it the *B. typhosus* hardly multiplied at all, and at the end of ten days had died out. Certain organisms in sewage seemed to have a deleterious action on the *B. typhosus*, hastening its extinction, viz. the *B. fluorescens liquefaciens* and *B. fluorescens stercoralis*. Russell and Fuller, subjecting the bacillus to the direct action of sewage, found the survival to range from three to five days.

In dry garden earth, according to Dempster,¹ the *Bacillus typhosus* does not live longer than eighteen days (Firth and Horrocks recovered it up to twenty-five days), and in peat it dies within twenty-four hours. In moist soil, however, the bacillus was still alive on the forty-second day. In an artificially dried soil it was not found alive after the seventh day.

Sidney Martin found that in moist sterilized soil kept at temperatures from 3° to 37° C., the *B. typhosus* maintains its vitality for upwards of fifteen months, but that in unsterilized soil it rapidly dies.²

Mair³ concludes that the typhoid bacillus can survive in natural soil in large numbers for about twenty days, and is still present in a living condition after seventy to eighty days, but that there is no evidence that it is capable of multiplying and leading a saprophytic existence in ordinary soil. He suggests that Martin's result (the rapid extinction of the bacillus in unsterilized soil) may be explained by the use of broth cultures for infection, the broth added leading

¹ *Med. Chirurg. Trans.* lxxvii., 1894, p. 263.

² *Reps. Med. Off. Loc. Gov. Board* for 1897-1900, p. 525.

³ *Journ. of Hygiene*, viii., 1908, p. 37.

to a multiplication of the saprophytes. Firth and Horrocks¹ similarly conclude that the typhoid bacillus displays no tendency to increase in numbers, nor to grow upwards or downwards in soil, though it may be washed by water through a thickness of 18 inches. They did not find that virgin or sewage-polluted soils showed much difference in these respects.

In a research on the conveyance of infection by the air, Germano² investigated the duration of life of the typhoid bacillus in dust, on clothing, and in faeces. In dust the typhoid bacilli always die quickly on desiccation (within three days), but when kept damp they live much longer. In dust and sand infected with typhoid stools much the same result was obtained. On clothing, however, even when dried, the bacillus retained its vitality for more than a month. He considers from these experiments that the conveyance of the typhoid infection *through the air*, at any rate for any distance, is very unlikely.

Firth and Horrocks, however, found the *B. typhosus* to be alive in soil dry enough to form dust for as long as twenty-five days, and consider that infective material can be readily transmitted from dried soil and sand by means of winds and air currents. Doubtless much depends on the degree of dryness of the substratum. From khaki drill and serge inoculated with cultures, the bacillus was recoverable for ten to twelve weeks, and for ten to seventeen days from the same materials fouled with enteric faeces.

Firth and Horrocks demonstrated that house-flies can convey enteric infective material from specific excreta or other polluted material to objects on which they settle or feed, and the Commission which investigated the prevalence of enteric fever in the Spanish-American war ascribed to

¹ *Brit. Med. Journ.* 1902, ii, p. 936.

² *Zeitschr. f. Hyg.* xxiv., 1897, No. 3, p. 403.

MANUAL OF BACTERIOLOGY

principal part in the dissemination of the disease. The influence of the possibility of fomites soiled with typhoid feces, conveying the disease was that of the blankets used in the South African war and brought to this country; the result was a rise to several cases of typhoid fever.

There has always been considerable discussion on the relation of sewer-gas to disease. In enteric fever, typhoid, typhus, and tonsillitis most physicians hold that the presence of sewer-gas is, to say the least, a predisposing factor. Some have considered that the specific organisms are present in the emanations from sewers, and this is the case occasionally. Thus Horrocks¹ in some experiments performed at Gibraltar, by pouring sewage containing infected with typhoid culture down drains, showed that specific bacteria present in sewage may be ejected into the atmosphere of ventilation pipes, inspection chambers, drains, etc. by (a) the bursting of bubbles at the surface of the sewage, (b) the separation of dried particles from the

long exposed to the sewer air are not so easily infected as those exposed for a short time.

Action of Heat and Germicides, &c.—The *Bacillus typhosus* in broth culture is killed by a temperature of 53°–54° C. in half an hour, and of 56°–60° C. in ten minutes. The germicidal action of various disinfecting agents is given in the table in Chapter XXII.

Wines and spirits have some germicidal action on the typhoid bacillus. Champagne destroys the bacillus in ten minutes, white wines in fifteen to twenty minutes, red wines in thirty minutes or thereabouts. If diluted with water, the germicidal action takes much longer to accomplish, and the acidity, not the alcohol content, seems to be the active factor.¹ Spirits, such as whisky or brandy, if diluted with not more than one to two times the volume of water, kill in ten to twenty minutes.

Anti-Typhoid Serum.—Attempts have been made to prepare an anti-typhoid serum by inoculating horses with increasing doses of typhoid bacilli, first killed (by heat, chloroform, &c.) and then living, but such sera have proved quite useless.

By far the most promising method is that of Macfadyen,² who injected horses with increasing doses of typhoid cell juice obtained by triturating the bacilli in the presence of liquid air. The serum acquires high bacteriolytic and agglutinating properties and possesses to a marked degree preventive and curative properties when tested on animals.

Chantemesse³ by cultivating a virulent strain of the typhoid bacillus in a special broth made with ox spleen, heating the culture to 55° C., centrifugalizing and injecting horses with the fluid, obtains a serum which he claims has

¹ Sabrazès and Marcandier, *Ann. de l'Inst. Pasteur*, 1907.

² *Proc. Roy. Soc. Lond. B.* lxxii., 1903, pp. 76 and 351; *Brit. Med. Journ.* 1906, i. p. 905.

³ *Trans. XIV. Internat. Cong. Hygiene and Demography.*

MANUAL OF BACTERIOLOGY

curative properties. Thus, among 5621 cases by the expectant method the mortality was 17 per cent. While among 1000 cases treated with this serum the mortality was 4.3 per cent. The patients receive very small doses of the serum, 5 or 6 drops, and the dose is given only two or three times. This dosage is quite different from that of an ordinary antitoxic or anti-microbial serum. Wright suggested that in reality *toxins* (and not *bacteria*) in the serum might be the active agents. Metchnikoff has accepted this view, and the treatment, therefore, would seem to be a vaccine one.

Typhoid Vaccine.—Wright first prepared an anti-typhoid vaccine by the following method.¹ A virulent *typhoid bacillus* (the virulence being kept up by intraperitoneal passage through guinea-pigs) is grown in peptone broth in flasks at 37° C. for from fourteen to twenty-four hours.

The flasks are then so heated that their contents will remain at for a few minutes, a temperature of 55° C. To obtain uniform toxicity the contents of several

toxicity. Leishman now cultivates for only forty-two hours, and the bacteria are killed by heating to 53° C. for one hour, the higher temperature having proved to be deleterious to the efficiency of the vaccine. The symptoms produced by the inoculation when first introduced were rather severe, but with the use of improved methods are now hardly appreciable. Two doses of the vaccine should be given, with an interval of seven to ten days between the two. The results so far obtained with anti-typhoid inoculation suggest that it is distinctly protective, but Wright's statistics were not considered by Karl Pearson to be sufficiently conclusive judged by statistical methods.

Inoculation is now being extensively practised, and we may hope shortly to have definite information as to its protective power. One of the latest results published is that of the 17th Lancers in India. This regiment, which was exposed to a severe epidemic of enteric, had 147 officers and men inoculated out of a strength of 509. There were 62 cases of enteric, with 11 deaths; all of these occurred among the inoculated with the exception of two, both of whom had refused a second inoculation and both recovered.

RELAPSES.

Various hypotheses have been advanced to account for the relapses which occur in typhoid and other diseases (e.g. Malta and relapsing fevers). Chantemesse and Widal¹ showed that if the *B. typhosus* is injected into an animal together with toxins of the *Streptococcus*, *B. coli*, or *Proteus*, its virulence is much enhanced. If, then, immunizing and bactericidal properties of the blood and tissues are but slightly acquired during the attack, an absorption of toxic substances from the alimentary tract may be sufficient to give the typhoid bacilli still present a fresh start, and so produce a relapse. This Sanarelli² was able to do experimentally. If an animal be injected with a sub-lethal

¹ *Ann. de l'Inst. Pasteur*, vi., 1892, p. 755.

² *Ibid.* vi., 1892, p. 721, and *ibid.* viii., 1894, p. 193.

MANUAL OF BACTERIOLOGY

B. typhosus, and, after recovery, be then injected with *B. coli*, or *Proteus*, the animal suffers from a fresh k emia, the typhoid bacilli still present revive and virulence, and the animal dies. Wright and Lamb related another hypothesis.¹ The organisms in typhoid, and relapsing fevers, are deposited in the spleen and organs, multiply and form colonies there, which become free from the bactericidal substances by the formation of a bacterial envelope. 'As a result of this the spleen will as a whole, less agglutinating substances than the blood. When the anti-bacterial substances in the lymph have increased to such an extent as to penetrate the non-anti-bacterial envelopes which surround these the production of toxins will be so diminished that the fever will fall. If, however, for some reason or other, even a colony escapes the full anti-bacterial power of the lymph, it may be, to being shut off in a capillary which has become blocked, or in some other part not freely washed by the lymph stream, the bacteria of this colony would multiply until the blood has become modified in such a way as to bring about a diminution of the anti-bacterial

these will each protect against the race with which the serum has been prepared, but may not against some other races. It may be, therefore, that in a typhoid infection, for example, a particular race of typhoid bacilli is in excess, and when the anti-bodies for this particular race have been formed in sufficient quantity the disease process comes to an end. There may, however, be present at the same time other races which have produced little of their specific anti-bodies; these then begin to grow and multiply, and a relapse ensues.

In the case of relapsing fever, the organism is probably a protozoon, and in this and other protozoan diseases relapses may coincide with developmental cycles of the parasite, e.g. in malaria.

CLINICAL DIAGNOSIS.

1. *Blood Cultures*.—3 to 5 c.c. of blood are withdrawn from a superficial vein with a syringe with aseptic precautions, and 0.5 c.c. of the blood so obtained is sown into each of several tubes containing 15 to 20 c.c. of sterile broth. The tubes are incubated at 37° C., and if organisms develop, these are isolated and examined culturally for the typhoid bacillus. Coleman and Buxton recommend the following culture medium: Ox-bile 90 c.c., glycerin 10 c.c., and peptone 2 grams. Distribute in small flasks, 20 c.c. in each, and sterilize. Each flask is inoculated with 2 to 3 c.c. of blood, incubated for eighteen to twenty-four hours, then streaks from each are made on to litmus lactose agar plates, which are incubated for a few hours. If the growth does not redden the medium and a typhoid-like bacillus is present, it is tested for agglutination with typhoid-immune serum.

2. *Agglutination Reaction*.—This is carried out by the microscopic or the macroscopic (sedimentation) method described at p. 172. Dilutions of 1 : 30 and 1 : 50 should be made, and some make a 1 : 100 in addition. The microscopic method is the more rapid. Various apparatus (agglutinometers) can be obtained, consisting of measuring devices and a supply of dead culture, with which the sedimentation test can be carried out by anyone, and seem to be satisfactory.

3. *Ophthalmalmo-Diagnosis*.—Chantemesse (*loc. cit.*) has devised a method analogous to the ophthalmalmo-diagnosis for tuberculosis

(p. 307). A virulent typhoid bacillus is grown on agar in plate bottles (fig. 14, p. 71) at 87° C. for eighteen to twenty hours. The growth is then washed off each bottle with 4 to 5 c.c. of distilled water, the combined washings are heated to 60° C. for half an hour and afterwards centrifugalized, and the deposit is collected and dried. The dry residue is triturated with NaCl (powder 3 grm., NaCl 1 grm.), and during the trituration sterile distilled water is added little by little to the extent of 100 c.c. per 8 grm. This suspension is heated to 60° C. for two hours, allowed to stand for two days, being heated on each to 60° C. for half an hour, the clear liquid decanted from the sediment and centrifugalized. The fluid is then mixed with ten times its volume of absolute alcohol, the precipitate allowed to settle, collected, and dried *in vacuo*. The dry substance is powdered in an agate mortar, and for use 8 to 10 mgrm. are dissolved in 1 c.c. of sterile water. Of this solution a drop is instilled into the conjunctival sac; and in a case of typhoid, after a lapse of two to three hours the conjunctiva becomes red and there is a sensation of heat, after six to ten hours there is a marked conjunctivitis which may persist for one to three days and then passes off. In healthy persons and other diseases no conjunctivitis ensues.

4. *Puncture of the Spleen with a sterilized hypodermic needle and syringe.*—A little of the blood and pulp is withdrawn with the syringe, and cultivations made as in 1. This method seems hardly justifiable, and now that the blood-culture method and agglutination reaction have been introduced should be discarded.

5. *Examination of Pus.*—Cultivations may be made as in 1 if the bacillus is present, apparently in pure culture. If not, plate cultivations, preferably on litmus lactose agar, Conradi-Drigalski agar, or malachite-green agar, may be prepared (see 'Water').

6. *Examination of the Stools.*—This is not a practicable method for clinical diagnosis, it takes too long, is tedious and uncertain. Plate cultivations from the diluted stools are made on Conradi-Drigalski, or on malachite-green, agar (see 'Water').

THE GÄRTNER OR ENTERITIDIS GROUP OF BACILLI.

The Gärtner group of bacilli, of which the type is the *B. enteritidis* of Gärtner, are bacilli morphologically

resembling the *B. typhosus*, i.e. they are pleomorphic, actively motile, multi-flagellate, non-sporing, and non-Gram-staining, but culturally are intermediate between *B. typhosus* and *B. coli*. Thus, like *B. coli*, they ferment glucose with the production of gas and acid and change neutral red; like *B. typhosus* they may not attack lactose and do not curdle milk; on potato they may form a thick yellowish growth like that of *B. coli*, or a thin, grey, invisible growth like that of *B. typhosus*. In litmus milk they usually first produce slight acidity, followed after three to four days by a change to alkalinity, and the milk ultimately becomes limp. The fermentation reactions of the chief members of the Gartner group are given in the table on p. 354. The organisms of the Gartner group may be divided into four sub-groups:

1. *Enteritidis group*.—Produce acute gastro-intestinal disturbance in man. The cause of epidemic meat-poisoning, e.g. the *B. enteritidis* of Gartner.

2. *Pneumonic group*.—Produce pneumonic symptoms in man. The cause of some outbreaks of epidemic pneumonia, e.g. *B. psittacosis*.

3. *Paratyphoid group*.—Produce a disease resembling typhoid fever in man. Subdivisions A and B.

4. *Group non-pathogenic to man*, e.g. *B. suicholerae* and *B. typhi murium*.

THE BACILLUS ENTERITIDIS.

A number of outbreaks of what has been termed 'epidemic meat poisoning' have been traced to infection with the *B. enteritidis*. The disease takes the form of an acute gastro-enteritis—urticaria, abdominal pain, vomiting, diarrhoea, nervous symptoms and collapse—occurring from eight to thirty-six hours after partaking of a meat meal, usually pork (sausage, pork-pie, ham), occasionally beef and tinned meat. The principal outbreaks of this nature

MANUAL OF BACTERIOLOGY

those at Jena, in 1888, investigated by Gärtner, which he isolated the type form of the *B. enteritidis*; Middlesborough, in 1888; in 1896; and Derby in 1902. A small outbreak occurred at Bedford in 1907.¹ These outbreaks are caused by varieties of the *B. enteritidis* of the general characters of the group, which may or may not² ferment lactose, and are distinguished by agglutination reactions, the organism isolated agglutinating well with the patient's serum.

B. enteritidis in morphology, motility, and staining resembles the *B. typhosus*, forms no, or only a little, indole, and changes neutral red to a fluorescent colour. Litmus milk after a faint acidity becomes alkaline and is converted into a thin watery translucent fluid without coagulation.

PARA-TYPHOID FEVER.³

seem to be the preferable designation for those micro-organisms that produce typhoidal symptoms.

Para-typhoid infections may simulate typhoid fever in almost every particular, but are usually mild and non-fatal. They sometimes occur in epidemics, may be spread by drinking-water, and occur in all parts of the world.

The para-typhoid bacilli are morphologically like the typhoid bacillus and are actively motile, but they ferment glucose with the production both of acid and of gas. A number of races have been isolated differing from one another in their source, rate of fermentation of glucose, action on milk, action on neutral red, and agglutination reaction, and are distinguished by the names of those who isolated them.

Two groups of the para-typhoid may be distinguished which have been termed *a* and *b* by Buxton. Group *a* produces less gas in glucose media than group *b*; with group *a* milk remains permanently acid; with group *b* it becomes alkaline after a transient acidity; and though group *a* changes neutral red to yellow, the red colour tends to return after three weeks or so, while with group *b* the yellow colour is permanent. That is to say, in their reactions group *a* is more closely allied to the typhoid bacillus than group *b*.

The fatal cases seem usually to have been infected with the *b* group. The fermentation reactions of some of the para-typhoid bacilli (Day, Schottmüller, and Brion and Kayser) are given in the table on p. 354.

As regards the agglutination reaction, the blood of the para-typhoid fever patient either does not agglutinate the typhoid bacillus or agglutinates it only in low dilution—e.g. 1 in 30 or 40, while it agglutinates the para-typhoid bacilli in far higher dilution—e.g. 1 in 100 or 200, or even higher, thus in Cushing's case, the patient's serum agglutinated the para-typhoid bacillus isolated from it up to 1 in 8000.

The diagnosis of para-typhoid fever would be based on (a) the agglutination reaction; (b) the isolation of a para-typhoid bacillus by cultures from the blood (p. 345).

SWINE FEVER.¹

Swine fever, or hog cholera (to be distinguished from swine plague and swine erysipelas, which see), is an infective disease of pigs, highly contagious, and causing considerable mortality. The duration of the affection is usually three to four weeks; the animals lie about, their temperature is raised, and they may suffer from cough and frequent respiration, and some lameness in the hind legs. Towards the end mucous diarrhoea is a prominent symptom. Post mortem, the large intestine is found to be ulcerated, the ulcers much resembling the typhoid ulcers of man, and according to Klein, pneumonia is commonly present, whence he termed the disease 'pneumo-enteritis.' McFadyean, however, from his own experience and that of the Board of Agriculture, considers pneumonia very infrequent. The lesions are very distinctive, and their most constant seat is the caecum and colon. The commonest form of the intestinal lesion is a well-defined circular necrosis involving the whole thickness of the mucous membrane and occasionally extending to the wall of the bowel. A diffuse diphtheroid lesion comes next in frequency, being in reality a superficial necrosis with deposition of a thin layer of fibrinous exudate on the surface. All gradations are found between the well-defined circular necrosis and the diffuse diphtheroid lesion.

An organism constantly present is a member of the Gärtner group (*B. suicholerae*), but it seems to be a terminal infection and not the true ætiological agent, as the blood and tissues filtered through a porcelain filter are still infective—i.e. the organism is

¹ See Uhlenhuth, *Trans. XIV. Internat. Cong. of Hygiene* (Berlin, 1907), 13d iv. p. 50.

probably ultra-microscopic. The *B. suicholera* is apparently identical with the *B. icteroides* of Sanarelli. (See also Chapter XX.)

Although the lesions are very similar, swine fever has nothing to do with typhoid fever of man, nor with ulcerative colitis.

Other organisms belonging to the Gärtner group are:

1. The Danysz bacillus used as a virus for exterminating rats (the Danysz virus).

2. The *B. icteroides* of Sanarelli, supposed by him to be the cause of yellow fever, but apparently identical with the *B. suicholera* (see 'Yellow Fever').

3. The *B. typhi murium* of Löffler, used as a virus for exterminating mice.

4. The *B. psittacosis* of Nocard, causing an infective disease of parrots and transmissible to man (bird-fanciers &c.), in whom it produces a severe and often fatal broncho-pneumonia.

5. *Summer Diarrhœa*.—Morgan¹ in cases of summer diarrhœa of infants has isolated a bacillus which appears to be most closely allied to the hog-cholera bacillus, differing from the latter by producing alkalinity in litmus milk (without previous acidity) and much indole, and by failing to produce acid and gas from arabinose, maltose, and dextrin.

BACILLUS DYSENTERIÆ.

In one type of dysentery, the so-called epidemic or bacillary form (see 'Dysentery,' Chap. XX.), a bacillus, *B. dysentericæ*, seems to be the causative agent. The *B. dysentericæ* includes a group of closely allied organisms.

The dysentery bacillus was first isolated from the stools in 1897 by Shiga in Japan. Somewhat later Kruse isolated an almost identical bacillus in Germany, and this type is known as the Shiga-Kruse type. During the last few years similar organisms, but differing from the Shiga-Kruse type in some of their fermentation reactions, have

¹ *Brit. Med. Journ.* 1906, i. pp. 908 and 1181; *ibid.* 1907, ii. p. 16.

been isolated; these are sometimes termed 'pseudo-dysentery' bacilli.

The Shiga-Kruse and other types of dysentery bacilli have been isolated by Flexner and Strong in the Philippines, Park, Duval, Bassett, Martini, Hiss, Russell and others in the United States, Castellani in Ceylon, Rogers and others in India, and Eyre, McWeeney and others in the British Isles.

Morphology.—The dysentery bacilli resemble the colon bacillus in morphology and staining reactions. They are non-motile, but Brownian movement is often active,¹ non-sporeing, and readily destroyed by heat (58°-60° C.) and antiseptics.

Cultural Characters.—The dysentery bacilli are aerobic and facultatively anaerobic. On agar a thinnish creamy growth develops; on gelatin a white growth nearly limited to the inoculation track, and without liquefaction. The colonies on a gelatin plate closely resemble those of the typhoid bacillus. On potato the growth is either thin, grey, and slightly visible, or thicker and yellowish or brownish. The colour of neutral red media is unaltered. Litmus milk first becomes faintly acid, then markedly alkaline; no clotting. Indole is generally not formed; occasionally a trace may be detected. All strains ferment glucose with the formation of acid only, no gas; none ferments lactose. Some strains (the Flexner type) ferment mannitol with the formation of acid only, no gas; other strains (the Shiga-Kruse type) have no action on this substance. The fermentation and other reactions are given in the table on p. 354, and are contrasted with those of the *B. typhosus*, *B. enteritidis*, *B. coli*, and other organisms.

Agglutination Reaction.—The agglutination reaction

¹ Flagella have been described by some observers, but cannot usually be demonstrated.

is given by the blood of patients suffering from the bacillary form of dysentery, but not by the amœbic form (unless a double infection be present, which occasionally is the case). The agglutination reaction is obtained in dilutions of 1 in 10 to 1 in 100, but may occur only with the particular strain causing the infection.¹ Thus by the agglutination reaction variations between different strains of the *B. dysenteriae* may be detected.

Pathogenic Action.—No characteristic lesions are produced in animals by administration of the dysentery bacillus *per os*. In man, cultures given by the mouth are stated to have induced a typical dysentery. Animals, such as rabbits, guinea-pigs and mice, are very sensitive both to living and to killed cultures; in fact, it is very difficult to immunize animals against the organism. Amounts of 0·1-0·2 mgrm. of an agar culture given intravenously or intra-peritoneally are fatal to these animals.

In man the organism is abundant in the bloody mucoid discharge from the bowel, and at an early stage is easy to isolate by means of Conradi-Drigalski agar plates, on which it forms small blue colonies; at a later stage (after two to three days) the other organisms in the bowel multiply to such an extent that isolation may become very difficult.

Toxins.—The filtrate of dysentery cultures (four to six weeks old) in a somewhat highly alkaline broth (broth just alkaline to litmus + 7 c.c. normal NaOH per litre) is markedly toxic, 0·1 c.c. being a fatal dose for a large rabbit.²

Anti-Serum and Vaccine.—The serum of horses immunized with the toxin, or with dead and then with living cultures, possesses marked antitoxic properties, and the use of this antitoxic serum has been successful in cases

¹ See Hewlett, *Trans. Path. Soc. Lond.* lv., 1904, p. 51.

² Todd, *Journ. of Hygiene*, iv., 1904, p. 480 (Bibbogg).

of acute bacillary dysentery. Shiga obtained a reduction in mortality from 22 to 7 per cent. by the use of serum in a severe epidemic.¹

When the disease has become chronic the use of a vaccine, consisting of a culture sterilized by heat, is sometimes beneficial. Castellani also suggests the use of a vaccine for prophylactic purposes.

Para-Dysentery Bacilli.—In the dysenteries of Ceylon, Castellani² has sometimes isolated dysentery bacilli nearly related to the Shiga-Kruse type, but showing differences from it in agglutination, persistence of acid reaction in litmus milk, and virulence; these he has termed 'para-dysentery' bacilli.

Asylums Dysentery and Summer Diarrhoea of Infants.—Both in America and in England some cases of summer diarrhoea of infants are found to be associated with the *B. dysenteriae*. The asylums or institutional dysentery, or ulcerative colitis, is also due to this organism, and the blood of patients gives the agglutination reaction.³ In both instances the *B. dysenteriae* present is of the Shiga-Kruse type.

BACILLUS COLI.

The *Bacillus coli*, or colon bacillus (*B. coli communis*), is an organism of considerable importance, both in connexion with the *Bacillus typhosus*, in pathological processes, and in water supplies as an indication of pollution. As its name implies, it is a constant inhabitant of the intestinal tract in man and animals, and is, perhaps, one of the most widely distributed organisms in nature. While the term 'colon bacillus' is applied to a fairly well-defined organism (the 'typical *B. coli*'), there are a number of allied organisms differing from the type in one or more characters—e.g. motility, indole production, fermentation

¹ On vaccine prophylaxis and serum treatment, see Dean, *Journ. Roy. Army Med. Corps*, ix., 1907, p. 572.

² *Journ. of Hygiene*, iv., 1904, p. 495.

³ Hewlett, *Trans. Path. Soc. Lond.* lv., 1904, p. 51.

reactions, rate and extent of milk curdling, &c.—and these varieties are said to belong to the 'colon group,' or are termed 'coliform.'

The *B. coli* may be readily isolated by inoculating litmus lactose bile salt peptone-water tubes with a trace of a suspension of fresh faeces, growing for twenty-four to forty-eight hours at 42° C., and plating the culture out on litmus lactose agar, on gelatin, or on Couradi-Drigalski agar, or by direct plating of the faeces suspension on the last-named medium (see also 'Water').

Morphology.—The typical form of the colon bacillus has the following characters :

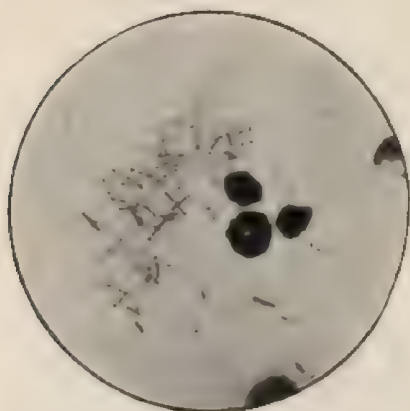
It is a short rod with rounded ends, 2 or 3 μ long and 0.4 to 0.6 μ broad, frequently linked in pairs or more. It is often so short that it is merely ovoid in shape ; and, on the other hand, longer individuals and involution forms occur 6 μ or more in length (Plate XII., b). It is feebly motile, and possesses lateral flagella to the number of three to four on an average, which are shorter and straighter than those of the typhoid bacillus (non-motile varieties are of frequent occurrence). It is sometimes met with in a diplococcoid form, which by cultivation in ascitic fluid may become fixed. An apparently capsulated form was described by Jenner.¹

Spore formation does not occur, but vacuolation may sometimes be observed. The organism is well stained by the ordinary anilin dyes, but is decolorized by Gram's method.

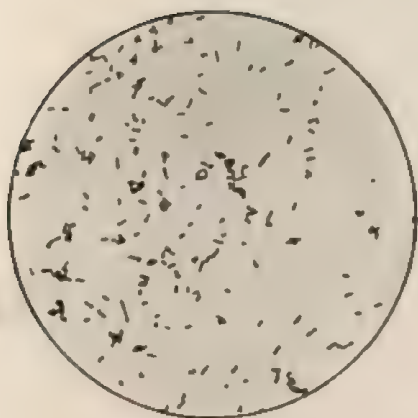
Cultural Characters.—The *B. coli* is aërobie and facultatively anaërobie, and grows readily on the ordinary culture media from 20° to 37° C. In gelatin plates the colonies are visible in twenty-four to forty-eight hours. The deep colonies are spherical, granular, and of a pale brownish colour, darker at the centre than at the periphery. The superficial colonies are at first punctate, round and almost transparent ; by rapidly spreading, they soon

¹ *Journ. Path. and Bact.* v., 1898, No. 3, p. 257.

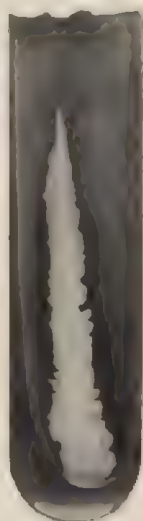
PLATE XII.



a. THE AGGLUTINATION REACTION.
A CLUMP OF TYPHOID BACILLI. $\times 1500$.



b. BACILLUS COLI.
COVER-GLASS PREPARATION OF A PURE
CULTURE. $\times 1000$.



c. GELATINE CULTURE OF
B. COLI, 6 DAYS OLD.



attain a diameter of 3 mm., and their margins become irregular, the surface is smooth, they are finely granular, opalescent in appearance, and thicker at the centre than at the periphery (fig. 41). On a gelatin streak a copious white, shining, smooth growth develops, the margins of which are irregular and crenated (Plate XII., c), and in old cultures the medium becomes opalescent. In a gelatin stab-culture a white growth develops along the line of

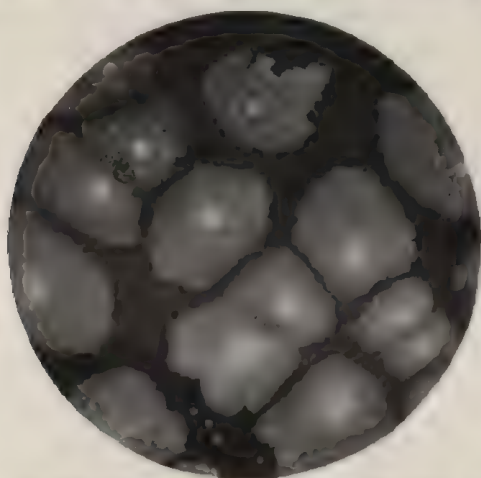


FIG. 41.—COLONIES OF THE COLON BACILLUS, SUPERFICIAL AND DEEP.

inoculation with one or more gas bubbles. The gelatin is not liquefied. On agar and on blood serum a thick, moist, shining, greyish layer forms. There is abundant formation of gas in a stab-culture in glucose-agar and in gelatin shake cultures (fig. 42), provided the medium be made with meat; 'lemco' gelatin, however, generally fails to give gas. On acid potato it forms a straw-yellow or brownish-yellow moist, thick growth, but if the potato is not fresh and acid in reaction the growth may be colourless. Milk is a good cultivating medium, and is curdled in twenty-four to

seventy-two hours. This curdling is principally due, not to an enzyme, but to the formation of a considerable amount of lactic acid, though a milk-curdling enzyme has been described by Savage¹ as being formed under certain conditions. The gas which is produced in culture media under anaerobic conditions consists of hydrogen and carbon



FIG. 42.—COLON BACILLUS. GELATIN SHAKE CULTURE
SHOWING GAS PRODUCTION.

dioxide. Under aerobic conditions marsh-gas is stated to be also formed. The ratio of H to CO_2 is about 2:1 for dextrose and lactose. In broth it produces a general turbidity without film formation, and the culture gives the indole reaction on the addition of a nitrite in twenty-four to forty-eight hours.

The fermentation reactions are given in the table, p. 354. It will be seen that the *B. coli* is an active fermenter of many carbohydrates, alcohols, and glucosides,² e.g. glucose, lactose, galactose, mannitol (but not dulcitol). Cane-sugar may or may not be fermented; sometimes only acid is formed, sometimes both acid and gas are produced. To

¹ *Journ. Pathol. and Bact.*, November, 1904.

² See Twort, *Proc. Roy. Soc. Lond. B.* lxxviii. p. 329.

the variety producing both acid and gas from cane-sugar Durham gave the name *B. coli communior*. Prescott and Winslow consider that the term *B. coli* should be applied only to an organism that does not attack ketonic sugars. Neutral red in glucose broth is changed to a fluorescent yellow, and Houston describes a typical *B. coli* as 'flaginac,' i.e. producing fluorescence in neutral red glucose-peptone water (fl), acid and gas from glucose (ag), indole in peptone water (in), and acid and curd in milk (ac). The colonies on Conradi-Drigalski agar are large and red (see 'Water'). The *B. coli* does not give the Voges-Proskauer reaction (p. 364).

The differentiation of the *B. coli* from the *B. typhosus* should present no difficulty if the morphology and motility of the organisms, and their fermentation and agglutination reactions, be compared. Bacteriologists usually make use of the following tests for the differentiation of *B. coli*: (1) morphology, (2) motility, (3) Gram staining, (4) character of growth and colonies on gelatin, (5) liquefaction of gelatin, (6) action on milk, (7) indole formation, (8) fermentation of glucose, (9) fermentation of lactose and saccharose, (10) action on neutral red. MacConkey suggests that instead of tests Nos. 4, 6, 7, 8, and 10, the following should be substituted: (a) fermentation of dulcitol, adonit, and inulin; (b) the Voges-Proskauer reaction.

Other media which have been recommended for the differentiation of *B. coli* from *B. typhosus* are the Proskauer-Capaldi media and Petruschky's litmus whey.

The Proskauer-Capaldi media have the following compositions:

Medium No. 1.

Asparagin	0.20 gram
Mannitol	0.20 "
Sodium chloride	0.02 "
Magnesium sulphate	0.01 "
Calcium chloride	0.02 "
Potassium mono-phosphate	0.20 "

These substances are dissolved in 100 c.c. of distilled water, and the solution is sterilized for an hour and a half in the steam sterilizer. The medium, which has a slightly acid reaction, is then carefully neutralized with caustic soda.

Medium No. 2 contains

Witte's peptone	2.0 grams
Mannitol	0.1 gram

The constituents are dissolved in 100 c.c. of distilled water, and the solution is sterilized in the steam sterilizer for an hour and a half. The medium, which has a slightly alkaline reaction, is carefully neutralized with a solution of citric acid. To each medium sufficient litmus solution is added to give a deep purplish-red colour, the media are again sterilized for half an hour, filtered, and filled into sterile test-tubes.

If these media be inoculated with *B. typhosus* and *B. coli* respectively and incubated at 37° C. for twenty-four hours, the following changes will be noted :

	<i>Medium No. 1.</i>	<i>Medium No. 2.</i>
<i>B. typhosus</i> . .	No growth or change in reaction.	Growth with strongly acid reaction.
<i>B. coli</i> . . .	Growth with acid reaction.	Growth with neutral or faintly alkaline reaction.

Petruschky's litmus whey is prepared as follows: Fresh milk is warmed and the casein precipitated by the addition of a minimal amount of hydrochloric acid. It is filtered, and the filtrate of clear whey is carefully neutralized with dilute caustic soda solution. The fluid is then steamed for two hours and filtered; the filtrate should be clear, colourless, and neutral in reaction. Enough neutral litmus solution is then added to render it well coloured, and the mixture is distributed into test-tubes and sterilized. This medium is rendered slightly acid (represented by 6-10 c.c. $\frac{N}{10}$ caustic soda per cent.) by *B. typhosus*, very acid (40-50 c.c. ditto) by *B. coli*.

The thermal death-point of the organism, according to Weisser and Sternberg, is 60° C. with an exposure of ten minutes. The *B. coli* will grow freely in a slightly acid medium, and in media containing as much as 0.15 per cent. of carbolic acid. In this respect it is a more resistant organism than the *B. typhosus*.

Chemical Products.—The acids produced are mainly lævo-lactic acid with some dextro-lactic acid from glucose, lævo-lactic acid only from mannitol; also acetic, formic and succinic acids, and alcohol. According to Harden, *B. coli* attacks glucose in a characteristic manner, each molecular proportion of sugar yielding half a molecular proportion of acetic acid and of alcohol, and one molecular proportion of lactic acid, together with a small amount of succinic acid, and gaseous carbonic acid and hydrogen. Nitrates are reduced to nitrites.

No toxin, or a trace only, is formed in cultures, but the dead bacilli are toxic and pyogenic, and a toxin is obtained by autolysis of cultures or by triturating the bacilli with liquid air (Macfadyen).

Vaughan,¹ by washing large quantities of colon and typhoid bacilli, extracting the bacterial cells first with alcohol, then with ether, and then digesting the ground residue with alcohol containing 2 per cent. NaOH, states that two constituents are obtained, one soluble in alcohol and toxic, the other insoluble in alcohol and non-toxic. The latter confers a certain degree of immunity on animals injected with it.

Pathogenicity.—The pathogenic action and pathogenicity of the *B. coli* are very varied. Introduced into the circulation in guinea-pigs it usually causes death in one to three days. Introduced into the peritoneal cavity in large amounts it produces death, apparently from a toxæmia. Some varieties are non-virulent to animals.

¹ Trans. XIV. Internat. Cong. Hygiene (Berlin, 1907), Bd. iv. p. 28.

In man the colon bacillus is associated with a number of important pathological processes. It is usually the organism causing the peritonitis which is due to infection from the intestine, as in hernia with obstruction or perforation, in ulceration of the bowel and enteritis, in cancerous growths, and affections of the appendix, biliary canals, and gall-bladder. The exudation in these cases is often characteristic; at first it is clear and greenish, it then becomes greenish-yellow, thin, semi-opaque and foul-smelling, and finally purulent. An important point is that the colon bacillus may pass through the intestinal wall where it has been damaged, as by strangulation, but not yet perforated.

The *B. coli* is a pyogenic organism, and has been met with in ischio-rectal abscesses (probably the *B. pyogenes fetidus* of Passet). Possibly it causes in some instances the pneumonia and pleurisy occurring after peritonitis, for it has been obtained from the lung and pleura in these conditions. Puerperal fever is another condition sometimes caused by the *B. coli*, and cystitis and infections after urinary operations are also commonly due to it.¹

In the Pictou cattle disease, characterized by extensive hepatic cirrhosis, Adami found a minute diplococcus or short bacillus. A similar form was afterwards isolated by him in hepatic cirrhosis in man. Miss Abbot,² from a study of several such cases, came to the conclusion that this organism was a variety of the *B. coli*. It has been suggested that hepatic cirrhosis is produced by poisons or toxins, e.g. of the *B. coli*, and that alcoholism, the usual cause assigned, is but an exciting or secondary agent.

¹ On the rôle of the *B. coli* in human infections, see Lartigau, *Journ. Amer. Med. Assoc.* April 12, 1902, p. 930 (full Bibliog.).

² *Journ. Path. and Bact.* vi., 1900, No. 3, p. 315 (Bibliog.).

Anti-Serum and Vaccine.—Attempts have been made to prepare an anti-serum for *B. coli* infections, but they have met with little or no success.

A vaccine prepared by sterilizing cultures by heat and standardizing has been used successfully in the treatment of chronic *B. coli* infections, e.g. cystitis. The *B. coli* vaccine is more toxic than most vaccines, and small quantities must therefore be given (see p. 199).

CLINICAL EXAMINATION.

1. The appearance and odour of the pus are often characteristic. Cover-glass specimens of the pus show small bacilli, which are decolorized by Gram's method.

2. The organism may be isolated by plating on gelatin, agar, litmus lactose agar, Conradi-Drigalski agar, or by the use of neutral-red or bile-salt media (see 'Water'). The isolated organism must be tested as to its morphology, motility, non-Gram staining, non-liquefaction of gelatin, indole production, curdling of milk, and fermentation of glucose, lactose, dulcitol, mannitol, etc.

3. An agglutination reaction may likewise be tried, but if negative is of little value, as there are so many varieties of the colon bacillus, and one variety may not be agglutinated by the specific serum obtained with another variety. A positive reaction must also be carefully controlled, as the colon bacillus is much more readily agglutinated by normal serum than is the typhoid bacillus.

VARIETIES OF *BACILLUS COLI*.

Organisms are frequently met with in faeces, manure, sewage, and polluted water which resemble the typical *B. coli* in many of their characters but which differ from it in certain particulars. Thus the colonies on gelatin, instead of being smooth, may be wrinkled; milk may be but slowly curdled (three to eight days); acid or gas production, or both, in sugars may be less marked than usual. These organisms are generally regarded as varieties of the *B. coli*, and are perhaps derived from typical *B. coli*.

There is, however, little evidence that *B. coli* can be transformed into such varieties, or that these varieties can be re-converted into typical *B. coli*.

ORGANISMS THAT HAVE BEEN REGARDED AS VARIANTS OF
B. COLI.

A number of organisms have been regarded as being varieties of the *B. coli* (consult table of fermentation reactions, p. 354).

1. *Bacillus cavicida* (Brieger).—This resembles *B. coli* in most of its characters, but was stated to be non-motile. MacConkey says it is motile.

2. *Bacillus neapolitanus* (Emmerich).—Isolated from the bowel in cases of cholera. It differs from *B. coli* by not being motile, and by fermenting cane sugar.

3. Gas-forming bacilli of Laser¹ and Gärtner.²

4. Aerobic bacillus of malignant œdema (Klein).

5. *Bacillus lactis aerogenes* of Escherich.—Found in the intestine of nurslings. Much like *B. coli*, but is non-motile. It differs from *B. coli* by not fermenting dulcitol, by always fermenting saccharose, and by giving the Voges-Proskauer reaction (see table, p. 354). According to Harden and Walpole,³ its action on glucose differs from that of *B. coli*, more alcohol being produced and formed at the expense of that part of the molecule of the sugar which in the *B. coli* fermentation yields acetic and lactic acids.

The Voges-Proskauer reaction is obtained by growing the organism in 2 per cent. glucose broth in a fermentation tube (fig. 16, p. 75) for three days and adding some strong caustic potash solution; on standing exposed to the air a pink colour develops. According to Harden and Walpole⁴ the reaction is probably due to acetylmethyl-carbinol, which in the presence of air and potash is oxidized into diacetyl, which then reacts with

¹ See *Centr. f. Bakt.* (1^{re} Abt.), xiii., 1893, p. 217.

² *Ibid.* xv., 1894, pp. 1 and 276.

³ *Journ. of Hygiene*, v., 1905, p. 488; *Proc. Roy. Soc. Lond. B.* lxxvii., 1906, p. 390.

⁴ *Proc. Roy. Soc. Lond. B.* lxxvii., 1906, p. 399.

some constituent of the peptone in the medium, giving the pink colour.

6. *B. cloacæ* (Jordan).—Met with in sewage. In its general characters it has considerable resemblance to *B. coli*, but it produces more gas (75 per cent.) from glucose and liquefies gelatin (4-5 to 30 days), and, like *B. lactis aerogenes*, it always ferments saccharose, does not ferment dulcitol, and gives the Voges-Proskauer reaction. (See table, p. 354.)

CHAPTER XI.

BUBONIC PLAGUE—CHICKEN CHOLERA—MOUSE SEPTICEMIA.

BUBONIC PLAGUE.

The bubonic plague was epidemic throughout Europe during the Middle Ages; in England in the fourteenth century it appeared as the Black Death, and in the seventeenth century as the Great Plague of London, while numerous other lesser visitations have been recorded. At present bubonic plague is practically pandemic. It seems to have been almost always endemic in Persia and Syria. The disease from time to time completely disappears from a district which it has decimated, to reappear again after a considerable interval, this has happened not only in Europe, but also in Persia, Syria, India, and China.

It is a severe febrile disease accompanied by delirium and great depression, which rapidly passes on to the typhoid state. If the patient lives, hæmorrhages may occur under the skin and from the stomach, bowel, and kidneys. On the second or third day in the majority of cases (70 per cent. or more) the glands in the axilla, inguinal, and other regions become enlarged, whence the disease derives its name.

In another type, the pneumonic form, the respiratory tract and lungs are attacked, and in a third, the septicæmic variety, the organism is generalized, though all varieties tend to become septicæmic shortly before death.

At the commencement and at the end of an epidemic the disease may assume an extremely mild type, the so-called 'pestis minor.'

Bacilli were first observed in this disease in the blood, buboes, and organs by Kitasato¹ in 1894. In the same year (1894) Yersin² was commissioned by the French Government to investigate the outbreak of bubonic plague at Hong Kong, and he described the bacillus met with in the buboes and its cultural and pathogenic properties very fully. This organism is known as the *Bacillus pestis*.

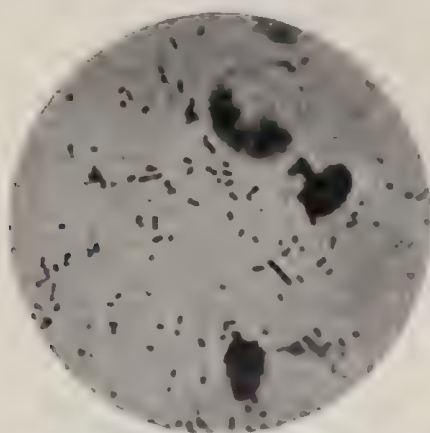


FIG. 43.—PLAGUE. SMEAR PREPARATION FROM SPLEEN OF INOCULATED GUINEA-PIG. $\times 1000$.

Morphology.—The *B. pestis* belongs to the group of hæmorrhagic septicæmic bacilli (rabbit septicæmia, swine plague, see p. 382), and is a markedly pleomorphic organism. *In the animal body* it occurs for the most part as a short, non-sporing, almost ovoid rod, generally linked in pairs, measuring $2-3\ \mu$ by $1-2\ \mu$, but longer forms may be seen

¹ *Lancet*, 1894, ii. p. 428.

² *Ann. de l'Inst. Pasteur*, viii., 1894, p. 662.

here and there measuring as much as $5\ \mu$ (fig. 43). Polar staining is a marked feature (Plate XIII., *a* and *b*). Occasionally swollen involution forms occur. The typical form of the organism, the bi-polar staining, short, stumpy bacillus is met with in smears from the buboes, in the sputum in the pneumonic form, and in the blood in the septicæmic variety, but only in the earlier stages of the disease. Later the typical forms tend to disappear, their place being taken by a few large, rounded, ovoid, or pear-shaped involution forms. Under cultivation the bacilli in young cultures (twenty-four to forty-eight hours) are so short as to be almost coccoid or slightly ovoid, on agar their size is about the same as that in the animal body, on gelatin they are somewhat smaller, but a few well-marked rods and even threads are always to be seen sparsely distributed in the preparations. In older cultures rod, thread, and involution forms occur more numerous; on agar containing 2-3 per cent. of salt the latter are swollen and yeast-like.

In broth the organism forms chains of slightly ovoid bacilli, much like a streptococcus (Plate XIV., *a*).

The organism is non-sporing and non-motile, although Gordon¹ has described the presence of one or two fine spiral terminal flagella.

Sometimes in hanging-drop cultivations a capsule is apparently present, but the writer has failed to verify this by staining methods.

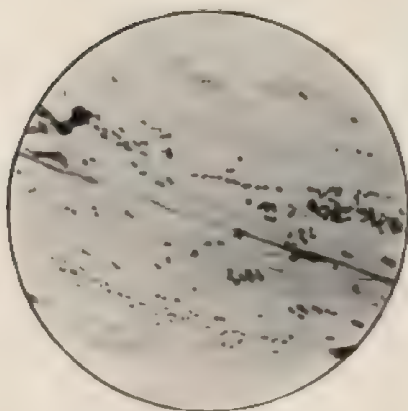
The *B. pestis* stains well with Löffler's blue and anilin-gentian violet, polar-staining being a marked feature, especially in smear preparations. It does not stain by Gram's method. With old laboratory strains, polar staining may be completely absent, but in such cases may sometimes be obtained by first treating the preparations with alcohol

¹ *Centralb. f. Bakt.* (1^{re} Abt.), xxii., 1897, p. 170. The Indian Plague Commissioners state that they have never been able to demonstrate the presence of flagella.

PLATE XIII.



a. *BACILLUS PESTIS*.
SMEAR PREPARATION FROM A BUBO. $\times 1000$.



b. *BACILLUS PESTIS*.
SMEAR PREPARATION OF SPUTUM. $\times 1000$.



or by the Gram method, and subsequently staining with Löffler's blue or weak gentian violet. Sections are best stained with carbol methylene or thionine blue.

Cultural Characters.—The *B. pestis* is aerobic and facultatively anaerobic. On blood serum it forms moist, smooth, shining, cream-coloured colonies or growths, slightly raised above the surrounding medium. The blood serum is not liquefied.

On agar the colonies are raised, round and cream-coloured, finely granular, denser at the centre than at the margins, which are regular. Size, 0·25 to 0·5 mm. in two days at 37° C.

On surface agar the *B. pestis* forms a thick, opaque, moist, smooth, cream-coloured growth, the margins of which are usually markedly crenated (fig. 44). The growth is very sticky and adherent. Haffkine states that when grown on *dry* agar (agar which has been kept in the warm incubator for two to three weeks) and viewed from behind the growth has an appearance like that given by the *back* of a mirror—i.e. a dull, silvery appearance.

On a salt agar (2·5–3·5 per cent. of sodium chloride) Hankin describes the development of remarkable spherical or pear-shaped involution forms.

On gelatin the colonies are whitish, filmy, finely granular with regular margins. Size, 0·1 to 0·25 mm. in five days at 22° C.

On surface gelatin the organism forms a thin, white, shining growth, with slightly irregular surface and margins, and nearly confined to the inoculation track. The



FIG. 44.—PLAGUE.
SURFACE CULTURE
ON GLYCINE AGAR,
48 HOURS OLD.

growth does not penetrate into the medium, nor does it render it cloudy. The growth is very adherent.

In a stab gelatin culture a delicate whitish, finely granular growth develops to the end of the stab, with little tendency to spread from the needle track. The gelatin is not liquefied. Both in agar and gelatin cultures fresh punctate growths sometimes develop in the original growth, simulating a contamination.

In broth the growth is somewhat characteristic. For two or three days the broth remains perfectly clear, but a flocculent growth forms and gradually increases in amount on the bottom and sometimes upon the sides of the tube. After some days the broth may become a little cloudy. A delicate, flocculent film develops if the tube be kept absolutely at rest. In broth to which a little butter-fat or ghee has been added little islands of growth appear on the surface, and from these flocculent tapering dependent growths form in about a week, provided the tubes or flasks be kept absolutely at rest, the bulk of the broth remaining clear. This is the stalactite growth of Haffkine, and is very characteristic (*B. pseudo-tuberculosis* and *B. suis-pestifer* also give it).

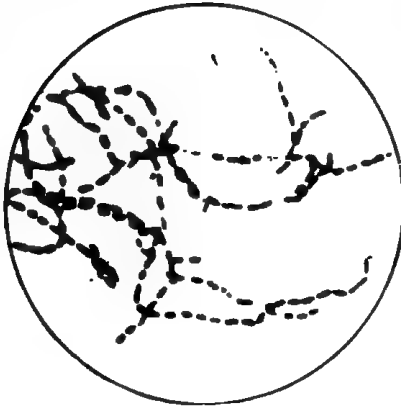
No growth occurs on ordinary potato, and milk is not coagulated.

With sulphuric acid alone a feeble indole reaction can be obtained with week-old broth cultures. With sulphuric acid and a nitrite a well-marked indole reaction can be obtained under the same conditions.

Broth cultures reduce a weak solution of methylene blue.

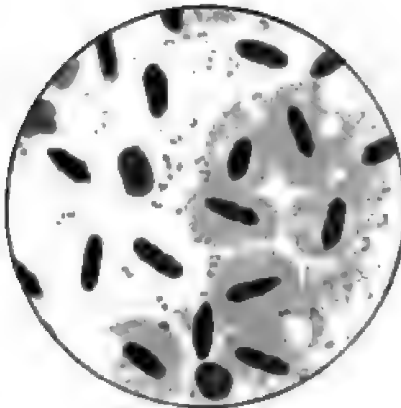
The fermentation reactions of the *B. pestis*, which MacConkey has pointed out are practically identical with those given by the *B. pseudo-tuberculosis*, are as follows: acid production but no gas in glucose, levulose, galactose, maltose, mannitol, and dextrin, no change in lactose, cane-sugar, and dulcitol.

PLATE XIV.



a. BACILLUS PESTIS.

COVER-GLASS PREPARATION OF A 48-HOURS' BROTH CULTURE. $\times 1500$.



b. CHICKEN CHOLERA.

COVER-GLASS PREPARATION OF BLOOD OF FOWL. $\times 1000$.



Action of Antiseptics, &c.—The plague bacillus is readily destroyed by antiseptics; a 1 : 1000 corrosive sublimate or 1 : 100 chloride of lime solution being efficient. An acid solution of corrosive sublimate is preferable, and for the practical disinfection of native houses a 1 : 250 solution of sulphuric acid may be employed. A temperature of 65° C. kills the organism in about fifteen minutes. Desiccation over sulphuric acid at 30° C. is also rapidly fatal.

Vitality and Virulence of Cultures.—Cultures retain their vitality for at least a month. As regards virulence, the organism differs much according to the source from which it is obtained. Under cultivation it gradually loses its virulence unless sub-cultured in the following manner: the cultures are made every week on agar, are placed in the blood-heat incubator for twenty-four hours, and then removed and kept at room temperature. If inoculated into animals, the virulence may be heightened for a particular species by successive passages, but in so doing is diminished for other species.

Pathogenic Action. In addition to man, the following animals are liable to contract plague under natural conditions—the monkey, cat, rat, mouse, squirrel, ferret, bandicoot, and marmot. The guinea-pig and rabbit are also susceptible to inoculation. The horse, cattle, sheep and goat are relatively insusceptible, though Simpson¹ has found that calves and poultry may be infected by feeding and suffer from a chronic form of the disease. Birds are not easily susceptible, and vultures feeding on the corpses of the plague-stricken do not seem to contract the disease. The mouse, rat, and guinea-pig are the animals chiefly used for experimental purposes in the laboratory; the first two are highly susceptible, a simple prick in the thigh with an infected needle being sufficient to induce the disease.

¹ *Report on the Plague in Hong Kong.*

here and there measuring as much as $5\ \mu$ (fig. 43). Polar staining is a marked feature (Plate XIII., *a* and *b*). Occasionally swollen involution forms occur. The typical form of the organism, the bi-polar staining, short, stumpy bacillus is met with in smears from the buboes, in the sputum in the pneumonic form, and in the blood in the septicæmic variety, but only in the earlier stages of the disease. Later the typical forms tend to disappear, their place being taken by a few large, rounded, ovoid, or pear-shaped involution forms. *Under cultivation* the bacilli in young cultures (twenty-four to forty-eight hours) are so short as to be almost coccoid or slightly ovoid, on agar their size is about the same as that in the animal body, on gelatin they are somewhat smaller, but a few well-marked rods and even threads are always to be seen sparsely distributed in the preparations. In older cultures rod, thread, and involution forms occur more numerous; on agar containing 2-3 per cent. of salt the latter are swollen and yeast-like.

In broth the organism forms chains of slightly ovoid bacilli, much like a streptococcus (Plate XIV., *a*).

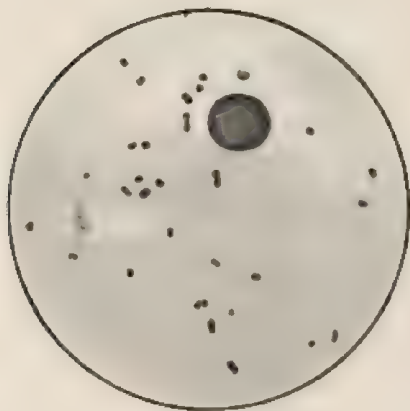
The organism is non-sporing and non-motile, although Gordon¹ has described the presence of one or two fine spiral terminal flagella.

Sometimes in hanging-drop cultivations a capsule is apparently present, but the writer has failed to verify this by staining methods.

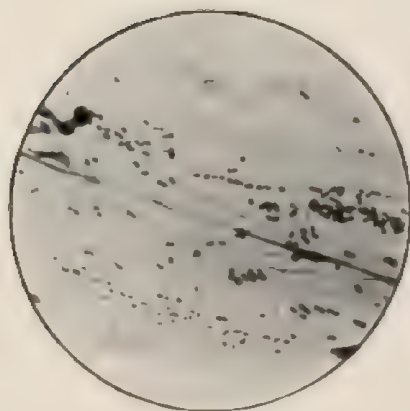
The *B. pestis* stains well with Löffler's blue and anilin-gentian violet, polar-staining being a marked feature, especially in smear preparations. It does not stain by Gram's method. With old laboratory strains, polar staining may be completely absent, but in such cases may sometimes be obtained by first treating the preparations with alcohol

¹ *Centralb. f. Bakt.* (1^{re} Abt.), xxii., 1897, p. 170. The Indian Plague Commissioners state that they have never been able to demonstrate the presence of flagella.

PLATE XIII.



a. *BACILLUS PESTIS*.
SMEAR PREPARATION FROM A BUBO. $\times 1000$.



b. *BACILLUS PESTIS*.
SMEAR PREPARATION OF SPUTUM. $\times 1000$.

mainly intracellular, but the broth itself is not without action.

Lustig and Galeotti¹ also devised an anti-plague vaccine, which is prepared by growing the bacillus on the surface of agar in dishes for three days, scraping off the growth, and treating with 1 per cent. caustic soda solution. The fluid is then filtered through paper and precipitated with very dilute acetic or hydrochloric acid, or by saturation with ammonium sulphate. The precipitate is dissolved in a 0.5 per cent. solution of sodium carbonate, and filtered through a Chamberland filter. This forms the vaccine fluid, which has the chemical characters of a solution of nucleo-proteins; it protects animals, and from the vaccinated animals an anti-plague serum can be obtained. The dose of this vaccine corresponds to 1-2 mgrm. of solid active substance. Monkeys vaccinated with 14-17 mgrm. of active substance become immunized so that they resist inoculation with virulent plague bacilli. Terni and Bandi suggested the preparation of a vaccine by inoculating rabbits intraperitoneally with plague bacilli, collecting the peritoneal exudation, diluting it with saline solution, incubating for a few hours, and then heating to 60° C. They claim that this vaccine produces a more lasting immunity than Haffkine's. Calmette² prepared a vaccine by growing the bacillus on agar in plate bottles (fig. 14, p. 71) for forty-eight hours at blood heat. Then 20 c.c. of sterile water are introduced, the growth is scraped off, the emulsion strained through a piece of fine sterile muslin to remove particles of agar, and the filtrate filtered through paper, which retains the microbes. These are well washed with sterile water to remove adhering toxin, next emulsified in sterile water, heated to 70° C. for an hour, and finally dried *in vacuo*. The dry substance can be kept for a

¹ *Brit. Med. Journ.* 1897, i. p. 1057, and 1900, i. p. 311.

² 'Harben Lectures on Plague,' *Journ. State Med.* 1900.

considerable time without change; for use 1-2 mgrm. are emulsified in 2-3 c.c. of sterile salt solution and injected.

Yersin proposed vaccinating directly with a plague bacillus of feeble virulence, which has been done by Strong in Manila. Though such a method might be used in a plague-stricken district, it is obviously one that could not be advised for general use.

Klein¹ has prepared a prophylactic by drying the organs of a guinea-pig dead of plague for three days at 46° C., rubbing the material to a powder, and further drying at 37° C. for three days. Of this dry powder 15-16 mgrm. protected a rat, and 25 mgrm. a monkey.

With reference to experimental immunity and protection in plague, Klein's experiments may be mentioned.² He found that a guinea-pig which had been three times injected with an amount of living culture insufficient to kill was still capable of being infected; that the blood of a guinea-pig which had twice passed through an attack of plague did not contain an appreciable amount of germicidal substances; and that the immunization of guinea-pigs by sterilized cultures is an extremely slow and difficult process. Calmette also found that the guinea-pig was extremely difficult to immunize.

Calmette has estimated the period of onset and duration of the immunity resulting from the injection of one, two, or three successive doses of plague culture killed by heat (i.e. Haffkine's prophylactic). The immunity induced by a single injection of 3 c.c. was not established until after the seventh day, and lasted on an average three weeks in the guinea-pig, a month in the monkey, and three months in the rat. By three successive injections at seven to eight days' interval a more durable immunity is obtained, extending to three to four months for the guinea-pig and monkey, and six months for the rat. While immunity

¹ *Rep. Med. Off. Loc. Gov. Board* for 1905-06.

² *Ibid.* 1896-97, App. B. p. 2.

is being established the animals are abnormally susceptible, and succumb to inoculation with minute doses of plague virus, usually non-fatal. As the outcome of these experiments, Calmette supposed that if a person in the incubation period of the disease were inoculated with the vaccine, a fatal issue would almost certainly ensue, and recommended that an injection of anti-plague serum (10 c.c.) should be given to induce a *rapid* protection, the Haffkine prophylactic being administered at the same time, the immunity produced by the latter being fully established when that of the serum is passing off. Bannermann,¹ however, strongly opposes the laboratory results obtained by Calmette. He finds that so far from there being an increase in mortality among those who have been inoculated and who develop plague within ten days of inoculation the reverse is the case, and that in a small community where the population has been partly vaccinated and partly not vaccinated, the incidence of plague during the week following vaccination was less among the vaccinated than among the un-vaccinated, pointing to the rapid production of protection.

Anti-Plague Serum.—Yersin was the first to prepare an anti-plague serum by inoculating horses intravenously with increasing doses of living plague bacilli. Roux afterwards immunized horses with killed cultures, but the serum thus obtained was not found to be so efficacious, so that now the living cultures are made use of. The exact method is briefly as follows—the agar surface in a plate bottle is inoculated, incubated at blood heat for 36-72 hours, the growth then emulsified by the addition of 20 c.c. of sterile physiological salt solution, and filtered through a little sterile cotton-wool to remove particles of agar. For the early injections the emulsion is heated to 65° C. for one hour, and the commencing dose is $\frac{1}{2}$ part of a flask. The

¹ *Centralb. f. Bakt.* (1^{re} Abt.), Bd. xxix. p. 873 (Bibliog.).

injections are given intravenously at intervals of a week. At the end of three months the bactericidal power of the blood will have become very marked, and living cultures are then injected for a further period of about three months until a whole flask is given at a dose. An interval of a fortnight is allowed to elapse between the last dose and the bleeding of the animal. The serum is tested upon the mouse; for immunizing power 0.05 c.c. should serve to completely protect a mouse against an inoculation of virulent plague twenty-four hours later, and for curative power, 0.25 c.c. should be sufficient to save the life of a mouse inoculated with plague sixteen hours previously. Anti-plague serum is mainly anti-microbic, and it is stated that as good a therapeutic or protective effect is obtained by injecting 0.5 c.c. of anti-plague serum together with 4.5 c.c. of *fresh* normal horse serum (i.e. containing active alexin) as by injecting 5 c.c. of anti-serum alone.

The anti-plague serum is not nearly so potent as the anti-diphtheria and anti-tetanus sera, and the earlier results obtained with it were by no means favourable. By using larger amounts and by giving the primary dose intravenously, Calmette and Salimbeni claimed to have obtained a reduced mortality of about 15 per cent. as compared with 60-70 per cent. for cases treated on general principles. Lustig and Galeotti have also prepared an anti-plague serum by inoculating horses with their vaccine, and with this serum Choksy has claimed to have had successful results.

Epidemiology. --The exact mode of infection in man has been a matter of controversy. Undoubtedly some lesion in continuity of the skin may be the point of entrance of the bacillus, which if arrested by the nearest lymphatic glands gives rise to the bubonic, if not so arrested to the generalized or septicæmic, form of the disease. The pneumonic form arises from infection by the respiratory

tract. Although a gastric and intestinal form of the disease has been described, and there is evidence to show that food or drink may be the vehicle of infection, this does not seem to be a common mode of infection. Yersin claimed to have isolated the bacillus from the dust and earth of a native dwelling, and Hankin from the brackish water in a field. Hankin's observations indicate, however, that infection is likely to occur only from immediate contact with man or animals, or their excretions, infected with plague, and not from a saprophytic form of the organism.

Certain animals, especially the rat, are important agents in spreading the disease. In many instances the association of sickness and of death among the rats with an epidemic of plague has been established. In some instances the epizootic among the rats definitely precedes the epidemic in man. The epidemics at Sydney are perhaps the most striking instances of rat-borne plague; discussing the first one Tidswell says: 'The one clear fact in our epidemic was that human beings were not becoming infected from one another.'¹ In the first epidemic the mode of introduction of the disease was never traced to any human source. During an epidemic the rats may be found in all stages of illness and the plague bacillus can be found in large numbers in their carcasses. In the various epidemics at Sydney, cases of plague first occurred among the rats and mice, followed after an interval of days or weeks by human cases. Other animals may also occasionally be the means of disseminating the disease.² The experiments of the Advisory Committee on Plague Investigation in India have conclusively shown the important part played by rats in the dissemination of the disease. The origin of the primary infection in rats is doubtful. They may possibly

¹ *Journ. Sanitary Institute*, xxi. Pt. iv. 1901, p. 549.

² Clemow, *Brit. Med. Journ.* 1900, i. pp. 1141 and 1216 (Bibliog.).

become infected from the dust or earthen floors of the native houses soiled with excreta or discharges of plague patients, or from their clothing, poultices, or dressings, but the readiest method is probably by feeding on the dead. Once the epizootic has started, further infection is simple; rats fight, and so may directly inoculate one another; the sick rats may soil grain or other foodstuffs, and the dead rats are eaten by their fellows. Moreover parasitic insects, especially fleas, undoubtedly may transmit the disease from one animal to another. Thus it is found that if guinea-pigs be placed in a plague-infected house, many of the animals contract plague; but if the animals be placed in cages of wire-gauze, the mesh of which is small enough to prevent access of fleas, the animals do not contract plague. The transmission of the disease from rats to man is more difficult of explanation. Persons handling rats dead of plague might readily be inoculated, but this cannot be the usual mode of infection. Ants, flies, mosquitoes, bed-bugs, pediculi, and fleas have all been suggested as means of disseminating the disease; the latter as directly transmitting the disease from the rat to man. Simond,¹ Calmette, and Tidswell supported this theory, but Galli-Valerio² opposed it; of its truth, however, there can now be little doubt. The European rat-flea (*Ctenopsylla musculi*) is probably not the usual agent of transmission, but the *Pulex cheopis*, the commonest flea found on rats in the Tropics. Rats in some instances suffer from chronic plague infection, and this may serve to bridge over the periods in the year when human plague does not occur. Whatever be the actual mode in which the disease is transmitted from the rat to man, the fact that it is transmitted is fully proved, and therefore extermination of rats, either by trapping, poisoning, or asphyxiating,

¹ Simond, *Ann. de l'Inst. Pasteur*, xii., 1898, p. 625.

² *Centralb. f. Bakt.* (1^{re} Abt.), xxvii. p. 1.

or by the use of the Danyesz rat virus (see p. 351), is one of the means to be adopted in fighting the disease.

On Plague, see Simpson, *Treatise on Plague* (Cambridge University Press); Klein, *Bacteriology of Oriental Plague*; *Reports on Plague Investigation in India, Journ. of Hygiene* (Extra Numbers), VI. No. 4, VII. Nos. 3 and 6, and VIII. No. 2; *Rep. of the Indian Plague Commission*; Numerous *Reports* published by the Indian Government.

CLINICAL EXAMINATION.

1. Withdraw a little of the fluid from the bubo by means of a syringe. Make cover-glass preparations and stain with methylene or thionine blue. Mount and examine. Search for short plump bacilli, often in pairs, with polar staining and transparent centres. They are not stained by Gram's method.

N.B. There may be a mixture of organisms in the buboes.

2. Make agar plates and broth cultures. From colonies on the agar plates the organism may be isolated and its cultural and pathogenic characters ascertained. The appearance of the broth cultures, if characteristic, would be very suggestive of plague, but if uniform turbidity develops this may be due to contaminating organisms, e.g. micrococci.

3. Inoculate mice, rats, or guinea-pigs subcutaneously with the fluid or with the culture. Some of the animals should be inoculated by the cutaneous method—rubbing a little of the material on the shaved abdomen. Inoculation of rats serves to distinguish the *B. pseudo-tuberculosis* from the *B. pestis*. If the animals die, investigate for the *Bacillus pestis* by staining and culture methods.

4. In the pneumonic form, dilute the sputum with a little boiled water, inoculate several agar tubes, and incubate at 22°–23° C. Examine in two to three days. Also daub the nostrils of a guinea-pig or rat with a brush or pledget of wool dipped in the diluted sputum, avoiding wounding the mucous membrane. Film specimens of the sputum may also be made, stained, and examined. Gram's method will distinguish the *B. pestis* from the *Diplococcus pneumoniae*; the latter stains well by Gram.

5. *Agglutination Reaction*.—The Indian Plague Commissioners state that in their opinion no practical value attaches to

the method of serum diagnosis in plague, but a modified method is considered by Dunbar¹ to be of considerable value. The method is carried out as follows :

A small quantity of peptone solution, inoculated with the tissue-juice from the suspected organ, is mixed with an equal quantity of plague-serum of such a strength that the dilution reduces it to 1 : 200 (approximately). A second dilution of 1 : 400, and a third of 1 : 800 are also prepared.

As a *control*, an equal quantity of the inoculated peptone water is mixed with normal serum (rabbit or horse serum), the dilution being 1 : 100.

In a few minutes a distinct difference is observable. The 'control' shows with the oil-immersion lens a few isolated non-motile bacteria, while the plague-serum dilution 1 : 200 shows larger and smaller masses of agglutinated bacteria.

After two hours' incubation the same result is obtained with the plague-serum dilution of 1 : 400. No agglutination, however, is observed after incubation for twenty-four hours of the dilution of 1 : 800. This agglutination reaction, in conjunction with other suspicious phenomena, justifies an official notification of suspected plague.

CHICKEN CHOLERA.²

Chicken cholera is a disease of poultry characterized by profuse diarrhoea ; its course may be very rapid, and the bird found dead without having shown signs of illness. The organism is a very short rod, non-motile, so short that it is almost ovoid, 0·6 to 0·8 μ in length, and 0·3 to 0·4 μ in diameter. It stains by the ordinary anilin dyes, but not by Gram's method, and the staining tends to be polar, so that Pasteur, who first investigated the disease, described it as a diplococcus (Plate XIV., *b*). The organism grows freely on the various culture media from 20° to 38° C. ; on agar forming a thick, moist, cream-coloured layer, on gelatin a shining, white, expansive growth without liquefaction. In broth a general turbidity forms ; but growth on potato is indifferent. It produces acid, does not ferment glucose or lactose, is aerobic and

¹ *Centralb. f. Bakt.* xli. (Originale), 1906, p. 860.

² See *Journ. Exp. Med.* iii., 1898, p. 651.

facultatively anaërobic, does not form spores, and is killed by a temperature of 60° C. in fifteen minutes. If dried it dies in a few days, but retains its vitality for a considerable time in damp earth or in water, and so infection is readily conveyed. Fowls die after subcutaneous, intramuscular, or intravenous inoculation and by feeding, the organisms being found abundantly in the blood. Post mortem, the serous membranes may be inflamed and hæmorrhagic, the liver large and soft, and the intestine shows hæmorrhagic spots, and is sometimes ulcerated and contains a mucoid fluid stained with blood. Other birds, pigeons, pheasants, sparrows, wild and domestic ducks are also susceptible to the disease, and rabbits and guinea-pigs can be successfully inoculated; in the latter animal a local abscess sometimes forms instead of a general infection. By continuous cultivation with free access of oxygen the virus becomes attenuated, and Pasteur was able thus to prepare a vaccine which protected fowls.

The bacillus of chicken cholera seems to be identical with Koch's bacillus of rabbit septicæmia, and with the bacillus of swine plague (*B. suispestifer*, Löffler and Schutz, Salmon and Smith). These organisms tend to form a stalactite growth in butter broth.

Organisms have been described by Klein in fowl enteritis, grouse disease, &c., differing somewhat from the bacillus of chicken cholera.

MOUSE SEPTICÆMIA.

This disease may be conveniently described here. Koch first obtained a minute bacillus by injecting putrefying material subcutaneously into mice. It seems to be identical with the bacillus found in swine erysipelas. The organisms are met with in large numbers in the blood and tissues of mice. They measure only 1 μ in length, and occur in considerable numbers in the leucocytes. The bacillus stains well by Gram's method, and is stated by some writers to be motile. It grows readily, forming on agar extremely delicate, almost invisible colonies; in stab gelatin cultures after some time a delicate cloudiness radiates from the central puncture. From an agar culture the bacilli are somewhat larger than those found in the animal body, and form filaments. It is pathogenic for swine, rabbits, and mice.

CHAPTER XII.

PNEUMONIA AND INFLUENZA.

PNEUMONIA.

Several types of pneumonia must be recognized clinically, and are associated with different organisms. The pneumonia accompanying or complicating many of the specific fevers is usually of the catarrhal or lobular type, and may be set up by their respective organisms, as in diphtheria, enteric fever, influenza, plague, &c. There is also the so-called 'septic pneumonia,' unfortunately only too common after operations about the mouth, and generally due to the *Streptococcus pyogenes*. In pyæmic conditions pneumonic complications are frequent, and are produced usually by emboli conveying the causative organism, which may be one of the pyogenic cocci, most commonly the *Streptococcus pyogenes* or the *Micrococcus pyogenes* var. *aureus*, or the colon bacillus. Lastly, we have the acute croupous or lobar pneumonia, which in many of its characters resembles an acute specific infection.

Friedländer in 1862-63 described an oval encapsuled micro-organism in cases of pneumonia, which he found experimentally caused hepatization of the lung in mice and guinea-pigs.

In 1883 '85 Talamon, Klein, and Sternberg each described in pneumonic sputum an oval organism, encapsuled like Friedländer's, which induced pneumonia in animals; it was termed by the former the *Micrococcus lanceolatus*, and by Sternberg the *Micrococcus Pasteuri*, and, although there were certain discrepancies, these observers believed it to be identical with Friedländer's organism. Subsequently, however, Sternberg recognized that the discrepancies were due to the fact that different organisms were being dealt with, a conclusion that was subsequently con-

firmed by the researches of Fränkel and Weichselbaum. Fränkel isolated from the rusty sputum of pneumonia an organism which he termed the 'micrococcus of sputum septicæmia,' which was not the same as, and was more constantly present in pneumonia than, Friedländer's organism, and which he found was identical with Talamon's and with Sternberg's organism. Weichselbaum, in an extended research, obtained Fränkel's organism, which he termed the *Diplococcus pneumoniae*, in a large proportion of his cases, and the bacillus of Friedländer in only a few.

There seems to be little doubt that the majority (95 per cent.) of cases of acute croupous pneumonia are dependent upon this *Diplococcus pneumoniae*, and that Friedländer's organism, now termed Friedländer's pneumococcus, or, better, pneumobacillus, is of ætiological significance in only a small minority, if at all. It is, however, associated with certain pathological processes which will be referred to below.

In pleuro-pneumonia of cattle, by growing material in broth in collodion capsules in the peritoneal cavity, Nocard and Roux¹ have succeeded in cultivating an organism which is of interest as being the smallest of visible organisms. It is only just visible as a minute refractile granule with a magnification of 2000 diameters.

THE DIPLOCOCCUS (STREPTOCOCCUS) PNEUMONIÆ.²

Synonymus, Fränkel's pneumococcus, *Micrococcus Pasteuri* (Sternberg), *Micrococcus lanceolatus* (Talamon), *Micrococcus pyogenes tenuis* (Rosenbach).

Morphology. — The *Diplococcus pneumoniae* in the sputum and tissues usually occurs as an oval or lance-shaped coccus united in pairs, occasionally in chains of three or four elements, and then often almost spherical, and is generally surrounded by a well-marked capsule (Plate XV., *a*). In order to isolate the organism several tubes of glycerin agar, serum or serum-agar may be inoculated with rusty sputum and incubated for forty-eight hours; in some a

¹ *Ann. de l'Inst. Pasteur*, xii. p. 240.

² On the pathology of pneumococcus infection see *Brit. Med. Journ.* 1901, ii. p. 760; Eyre, *Lancet*, 1908, i., Feb. 22.

pure culture may be obtained. A far more certain method is to inject a drop or two of the rusty sputum into the peritoneal cavity of a mouse or rabbit. The animal will die in from twenty-four to thirty-six hours, and the organism will be found in considerable numbers in the lung and blood, from which cultures may be obtained. It is non-motile, stains with the ordinary anilin dyes, and by Gram's method.

Cultural Characters.—The *D. pneumoniae* is aërobic and also facultatively anaërobic. On glycerin agar and on serum at 37° C. it forms minute, transparent, almost invisible colonies like droplets of fluid; on serum the growth has much the same characters, but is somewhat more abundant. It hardly grows on gelatin at the ordinary temperature, but in a 120 per cent. gelatin at 25° C. minute white colonies develop without liquefaction. In broth it produces a slight cloudiness; it does not grow on potato, but develops in milk, which is usually coagulated; neutral litmus glucose-agar becomes red during growth, indicating the production of acid. The fermentation reactions are given in the table on p. 214. Hiss's medium (p. 268) with inulin is fermented and coagulated; most other streptococci fail to ferment inulin. On the ordinary culture media it retains its vitality for a short time only, not more than about a week; but if a little blood be smeared over the surface of the agar the vitality may be prolonged for a month or even longer. Washbourn recommended an agar rendered alkaline to the extent of 4 c.c. of normal caustic soda per litre, after neutralization, rosolic acid being the indicator. This medium is smeared with blood, placed in the incubator for twenty-four hours to ascertain whether it be sterile, then inoculated, capped, and kept at 37° C. Foa's method for keeping Frunkel's pneumococcus alive and virulent is to receive the infected blood of an inoculated animal into a small glass tube 5 mm. in diameter and 20 cm. long, so that

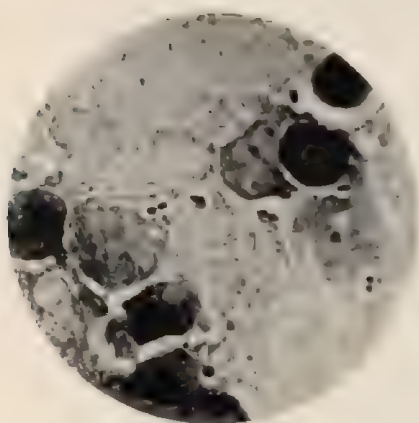
the blood completely fills the tube, which is then sealed and kept away from the light at the ordinary temperature.¹ If inoculated on to ordinary gelatin, which is then kept in the *blood heat* (37° C.) incubator, the organism retains its vitality for a month or six weeks.

Under cultivation the *D. pneumoniae* usually assumes the form of a short streptococcus (Plate XV., *b*) (included by Gordon in his *S. brevis* class) and the capsule is lost, but is regained again on passage through a susceptible animal, or by growing in fluid serum. A good deal of variation occurs in the morphology of the organism obtained from different sources and under cultivation. The thermal death-point of the *D. pneumoniae* according to Sternberg is 52° C., the time of exposure being ten minutes, and it is readily destroyed by the ordinary germicides, by light, and by desiccation; but in dried sputum it may retain its vitality and virulence unimpaired for weeks.

Pathogenic Action.—The *D. pneumoniae* is pathogenic for a number of animals, the most susceptible being mice, then in decreasing order, rabbits, rats, guinea-pigs, and dogs. Pigeons and fowls are immune. Death follows from subcutaneous, intravenous, intraperitoneal, or intrathoracic injection of a virulent culture, or of rusty pneumonic sputum, into mice and rabbits in twenty-four to forty-eight hours. The virulence of the organism varies considerably; under cultivation it may be completely lost, while by a series of passages through a susceptible animal it may be much increased. The less virulent it is the longer it tends to retain its vitality under cultivation. Except when injected into the lung or into the trachea, pneumonia does not result, but the disease runs the course of a septicæmia with high temperature and dyspnoea, death being generally preceded by a subnormal temperature and often convulsions. The post-mortem appearances are much oedema and inflam-

¹ *Brit. Med. Journ. Ept.* 1896, i. p. 68, No. 324.

PLATE XV.



a. DIPLOCOCCUS PNEUMONIE.

COVER-GLASS PREPARATION OF BLOOD OF INOCULATED ANIMAL. $\times 1000$



b. DIPLOCOCCUS PNEUMONIE.

COVER-GLASS PREPARATION OF A PURE CULTURE. $\times 1500$.



matory infiltration at the seat of inoculation, hæmorrhages in the serous membranes, enlargement and congestion of the spleen, and congestion of the lungs. The organisms occur in large numbers in the blood, lungs, and spleen, usually in the form of oval diplococci with well-marked capsules, but sometimes as short chains of streptococci. When injected into the lung or trachea a typical fibrinous or croupous pneumonia results.

The *D. pneumonia* is the cause of acute croupous pneumonia in man, and occurs in large numbers in the rusty sputum and hepatized lung, and in 20 per cent. of the cases can be isolated from the blood if 5-10 c.c. be cultured. The production of a typical pneumonic process experimentally and the presence of the diplococcus in a large proportion of cases of acute croupous pneumonia point to its specific relationship to the disease. With regard to the latter observation, Weichselbaum obtained it in 94 cases out of 129 examined, Wolf in 66 out of 70 cases, and Netter in 75 per cent. of the cases examined. In America the disease has of late been much on the increase, in Chicago the mortality having reached as high as 20 per 10,000 inhabitants. Acute croupous pneumonia sometimes occurs in epidemic form.

The organism is frequently present in the saliva of healthy individuals, as shown by Netter, Sternberg, and others, and the generally accepted idea of the relationship of 'catching cold' to an attack of the disease is explicable on the theory that the action of cold lowers vitality, and renders the tissues vulnerable to the attacks of the organism already in close proximity to them.

Besides acute croupous pneumonia, more than half the cases of broncho-pneumonia, both primary and secondary in the course of other diseases, are due to the *D. pneumonia*, which is also associated with a number of other important pathological conditions in man. It is a pyogenic organism, producing abscesses when inoculated into an insusceptible

animal such as a dog, and has been met with in abscesses, empyema, suppuration in the antrum, and purulent arthritis.¹ It is also found in about half the cases of purulent meningitis, sometimes in cerebro-spinal meningitis, in about a third of the cases of otitis media and infective endocarditis, sometimes in purulent pericarditis,² and occasionally in peritonitis.

Toxins.—Auld³ separated a proteose and an organic acid from the blood and organs of infected animals, and from cultivations of the *Diplococcus pneumoniae* in alkali-albumin the same products were apparently obtained, the alkaline medium soon becoming permanently acid. The proteose on subcutaneous or intravenous injection produced some fever; on intrathoracic injection fever and dyspnoea, and post mortem pleurisy and consolidation of the lung were found. The organic acid produced slight rise of temperature, but no other symptom. Macfadyen⁴ obtained an endotoxin by triturating cultures with liquid air.

Anti-serum.—Immunity can be conferred on susceptible animals by treating them with attenuated cultures, and also by inoculation with increasing doses of filtered broth cultures of the virulent organism. G. and F. Klemperer used recent broth cultures heated to 60° C. for one or two hours. Washbourn used filtered cultures in defibrinated blood, 20 c.c. of which injected subcutaneously in a rabbit conferred immunity against virulent cultures, an immunity persisting for fifty or sixty days. The blood serum of such immunized animals will protect other animals when injected, and Klemperer, Issaef, and Washbourn have prepared a pneumonic anti-serum. The latter, by first immunizing a horse with filtered cultures, increased the immunity by injection with gradually increasing doses of

¹ *Brit. Med. Journ.* 1901, ii. p. 1803.

² *Still, Brit. Med. Journ.* Sept. 7, 1901.

³ *Brit. Med. Journ.* 1897, i. p. 775.

⁴ *Ibid.* 1906, ii. p. 776 (Refs.).

living virulent cultures, until a very high degree of immunity is obtained. This anti-serum has been used in the treatment of pneumonia and other pneumococcic infections, but the results have not been very encouraging. The protective serum seems to produce aggregation of the cocci when added to a culture of the diplococcus. Klemperer and Washbourn found that the serum of convalescent patients possesses some degree of protective power. The serum, however, taken during the pyrexial stage of the disease rather increases the susceptibility of animals to pneumococcic infection.

Vaccine.—A vaccine prepared from cultures killed by heat and standardized has been found of service in chronic pneumococcic infections, and its use has also been suggested in acute infections (p. 199).

FRIEDLANDER'S PNEUMO-BACILLUS.

This organism, already referred to above in the general discussion of pneumonia, and originally believed by Friedländer to be the cause of the disease, has been obtained by recent observers in only a small proportion of cases.

Morphology.—The *B. pneumoniae* is a very pleomorphic organism, occurring in sputum or in the blood of an inoculated animal generally as a short rod with rounded ends surrounded by a marked capsule. It is non-motile, does not form spores, and is readily stained with the ordinary anilin dyes, but not by Gram's method, an important distinction from the *Diplococcus pneumoniae*. In cultivations, it forms short rods, long rods, chains, and even filaments, the capsule being absent, but this is regained on passage through a susceptible animal.

Cultural Characters. The *B. pneumoniae* is aerobic and facultatively anaerobic, and may produce indole. It grows readily on the various culture media from 20° to 37° C., on agar and blood serum forming a copious, viscid,

greyish growth; on gelatin, a thick, white, shining, porcelain-like growth without liquefaction; and in stab-cultures in gelatin, a so-called nail-shaped growth is developed (fig. 46), consisting of a white growth along the needle track, tapering from above downwards, and at the surface heaped up and expanded, forming the 'head' of

the nail. On potato a copious whitish growth develops, while milk is curdled and gas bubbles frequently form in stab-gelatin cultures. It is an active fermenter of carbo-hydrates, and the fermentation reactions are given in the table, p. 354.

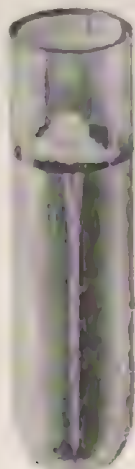


FIG. 46.—FRIEDLÄNDER'S PNEUMO-BACILLUS. GELATIN STAB-CULTURE, 7 DAYS OLD.

Pathogenic Action.—The pneumobacillus of Friedländer is pathogenic to mice and guinea-pigs, but rabbits are immune. Post mortem, the spleen is enlarged, the lungs are congested and consolidated in patches, and the organism is found in large numbers in the blood. It is possible that this organism may occasionally set up a pneumonic or bronchitic process in man, but this is doubtful. According to Curry¹ acute lobar pneumonia is

invariably due to the *D. pneumoniae*, though in a small percentage of cases Friedländer's bacillus may be associated with the former organism. It seems to be occasionally associated with anginal conditions, which are characterized by the formation of a false membrane with an absence of any general symptoms.² A microscopical examination of the membrane will show the organism surrounded with a capsule

¹ *Journ. Exp. Med.* iv., 1899, p. 169 (Bibliog.).

² *Ann. de l'Inst. Pasteur*, xi., 1897, p. 67 (Nicolle and Hébert). See also Pakes, *Brit. Med. Journ.* 1897, i p. 715.

and unstainable by Gram's method. If a culture be made on serum, the large, round, greyish colonies of the bacillus will be recognizable in fifteen to twenty hours, and should be examined microscopically. To obtain a pure culture a white mouse should be inoculated from a colony; it will die in twenty-eight to sixty hours. Friedländer's pneumo-bacillus has also been met with in water by Grimbert.¹ According to him it is identical with the *B. capsulatus* of Mori.

CLINICAL EXAMINATION (PNEUMONIA).

1. Make cover-glass specimens from the rusty sputum and stain some with Löffler's blue, and others by Gram's method with eosin. By a microscopical examination the oval diplococci will be readily recognized, Friedländer's pneumo-bacillus being distinguished from the *Diplococcus pneumoniae* by being decolorized by Gram's method. The latter organism is the only one, moreover, which is likely to be ordinarily met with.

2. If the diplococci are found to be fairly abundant in the sputum, and other organisms nearly absent, an attempt may be made to cultivate by inoculating several glycerin-agar and serum tubes and incubating at 37° C. for forty-eight hours.

3. If the diplococci are scanty, or so mixed with other organisms that it is difficult to identify them and probably impossible to obtain a pure culture, a drop or two of the sputum should be injected into the peritoneal cavity of a mouse or rabbit. The animal will die in from twenty-four to thirty-six hours, and the *Diplococcus pneumoniae* will be found plentifully in cover-glass specimens prepared from the blood or lung-juice, and pure cultures can be readily obtained by inoculating glycerin-agar tubes with the blood or lung-juice.

4. The culture or inoculation method, preferably both, will probably have to be adopted for the recognition and isolation of the *Diplococcus pneumoniae* in pus from empyemata, abscesses, &c.

5. Friedländer's pneumo-bacillus can be readily isolated by making gelatin plate cultivations, in which its colonies form white, shining, heaped-up points.

¹ *Ann. de l'Inst. Pasteur*, x., 1896, p. 708.

MANUAL OF BACTERIOLOGY

INFLUENZA.

minute bacillus was first described in this disease by in 1892, who found it in large numbers in the al secretion. In order to isolate the organism a with bronchial expectoration should be chosen ; he his mouth and gargles his throat with hot water times, and then, after coughing, the expectoration ined. A little of this expectoration is washed by g in a test-tube with sterile salt solution, then ng the washing with sterile salt solution in a second ally in a third test-tube. By means of a platinum a number of glycerin and blood agar tubes are ted with the sputum after the last washing, and ed at 37° C.

morphology.—The influenza bacillus is one of the t bacilli with which we are acquainted. It is a rod 0.5–1.5 μ in length, and is non-motile and non-

It soon dies out in cultivation, but according to Klein can be kept alive for some weeks in gelatin incubated at 37° C. The liquefied gelatin remains clear, the growth forming a delicate flocculent precipitate at the bottom. Preparations from cultures show long twisted chains and threads of bacilli, aggregated so as to form dense networks and convolutions. These chains or threads are composed of bacilli placed end to end, and united by a continuation of the cell-membrane. Involution forms occur. It is stated to grow better in association with the *M. pyogenes*, var. *aureus*, than alone. The organism does not seem to be able to live outside the body for any length of time, and is readily destroyed by desiccation, weak antiseptics, and by a temperature of 60° C. acting for five minutes.

Pathogenic Action.—Canon stated that he obtained this bacillus from the blood in a number of cases, but many other investigators have failed to find it. Klein also obtained it in six cases out of forty-three examined. According to Pfeiffer the bacillus is pathogenic only to monkeys and rabbits. Klein, however, was unable to obtain any definite effects in these animals by the injection either of sputum rich in bacilli or of pure cultures.

The influenza bacillus is met with in all uncomplicated cases of influenza in the nasal and bronchial secretions, often almost in pure culture, and in the bronchial tubes and lung in the pneumonic complications accompanying the disease. The organisms disappear with convalescence, and are not met with in other diseases. Klein¹ appears to consider that the pneumonia often complicating the disease is probably directly due to the bacillus. The typical influenza pneumonia is of the lobular type with a cellular rather than a fibrinous exudate. True lobar pneumonia,

¹ 'Further Report on Epidemic Influenza, 1889-92,' *Local Gov. Board Report*, 1893, p. 85.

due to the *Diplococcus pneumoniae*, may, however, complicate the influenzal attack. The organism also occurs in bronchitis, broncho-pneumonia, and whooping-cough.

Although the typical influenza may be due to the *B. influenzae*, many febrile conditions attended with pulmonary catarrh and frequently termed 'influenza' are not due to this organism. In an epidemic simulating influenza occurring in Essex in 1905, the examination was negative as regards streptococci, *B. diphtheriae*, and *B. influenzae*, but the *M. catarrhalis* was present in numbers in most cases (twenty-two out of twenty-four). This organism was originally isolated by Seifert in a small epidemic of infectious bronchitis, afterwards by Pfeiffer in cases of broncho-pneumonia in young children (see p. 224). Two other Gram-negative cocci were also isolated from three other cases (see table, p. 225).

CLINICAL EXAMINATION.

In cases of influenza, accompanied with bronchitis or pneumonia, the influenza bacillus may be met with in large numbers in the sputum, and their presence may aid in confirming the diagnosis. Cover-glass preparations may be stained with carbol-methylene blue.

CHAPTER XIII.

ANAEROBIC ORGANISMS.

TETANUS—MALIGNANT OEDEMA—BLACK QUARTER—BACILLUS
WELCHII (AEROGENES CAPSULATUS, ENTERITIDIS SPORO-
GENES)—BACILLUS CADAVERIS SPOROGENES—CLOSTRIDIUM
BUTYRICUM.

TETANUS.

The causation of tetanus was for a long time involved in mystery. No obvious or characteristic changes being met with after death, the disease was regarded by many as 'functional.' Others believed that a primary lesion of the central nervous system might be the cause of the affection, while a few classed it with the specific diseases.

It had long been noticed that wounds soiled with earth were specially prone to be followed by tetanus, and Sternberg in 1880, and Nicolaier in 1884, produced tetanus in rabbits by introducing a little garden earth beneath the skin. The latter observer found at the seat of inoculation and in his impure cultures—for he was unable to obtain pure ones—a distinctive bacillus, and he was able with these cultures, and with the pus from the seat of inoculation, to induce tetanus in other animals. Carle and Rattone subsequently showed that the bacillus of Nicolaier was present in the tissues of, and secretions from, the wound, in cases of traumatic tetanus in man, and that inoculation with the pus from such a wound produced tetanus in the lower animals, observations which were confirmed by

Rosenbach in 1885. The bacillus was isolated in pure culture by Kitasato in 1889, by taking the impure cultures obtained from the wound in a case of traumatic tetanus, heating to 80° C., and plating the heated cultures, the plates being incubated anaerobically in hydrogen.

Morphology.—The *Bacillus tetani* is a straight, slender rod with rounded ends, but under cultivation the rods may grow into longish filaments. It is somewhat motile and possesses a large number of flagella, three or four of which are generally thicker than the rest.¹ Spores are freely formed; they are spherical and develop at one extremity of the rod, and their diameter being much greater than that of the rod, the spore-bearing organism has been likened to a 'pin' or 'drum-stick' (Plate XVI., a). It stains with the ordinary anilin dyes, and also by Gram's method.

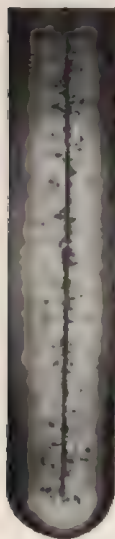


FIG. 47. — TETANUS BACILLUS. STAB CULTURE IN GLUCOSE AGAR, 7 DAYS OLD.

Cultural Characters. The *B. tetani* is a strictly anaerobic organism and will not grow in the presence of a trace of free oxygen, nor in an atmosphere of carbon dioxide. It can be cultivated in deep stabs in glucose agar and gelatin, or in broth by Buchner's method, or in an atmosphere of hydrogen (p. 64). In a gelatin stab-culture at 22° C. the growth radiates from the central puncture, and the gelatin is slowly liquefied. In a glucose agar stab-culture, it forms feathery, radiating outgrowths from the central puncture, a small amount of gas being formed (fig. 47). Broth becomes turbid with the formation of some gas and the development of a foul odour;

¹ Kanthack and Connell, *Journ. Path. and Bact.* iv., 1897. p. 452.

there is no film formation. The colonies have a central opaque portion surrounded by diverging rays. It grows on serum without liquefaction and in milk without curdling. The tetanus bacillus remains alive for some time in cultures, and the spores retain their vitality for months in the dried state, withstand a temperature of 80° C. for an hour, but are killed by boiling for five minutes. Carbolic acid (1 : 20) does not destroy the spores under about fifteen hours.

Pathogenic Action.—Man and the horse are most subject to tetanus; cattle and sheep are rarely affected, while the fowl, frog, triton, snakes, and tortoise are immune. Mice, rabbits, and guinea-pigs are all very susceptible. The bacillus is present in the superficial layers of the soils in many localities, but not in all, and this accounts for the fact that tetanus is rare in some places and frequent in others. Curiously enough, some of the savage inhabitants of the Solomon Islands have made use of poisoned arrows, the poisonous nature of which is due to tetanus-bearing earth. The arrows are tipped with a viscid fluid, then rubbed in the soil from a mango swamp, and afterwards dried. Individuals wounded with these arrows often develop tetanus.

The bacillus is confined to the seat of inoculation, or at most is met with in the nearest lymphatic glands, so that the general symptoms are due to the absorption of the chemical products. The organisms associated with the tetanus bacillus in earth are probably of considerable importance in the production of the disease, for it has been shown that if the tetanus bacilli and their spores be carefully washed so as to remove all adherent toxins, they fail to set up tetanus on inoculation, while if the same washed bacilli be injected, together with a little lactic acid, tetanus follows; the explanation being that the bacilli are unable to multiply unless the surrounding tissues are damaged and

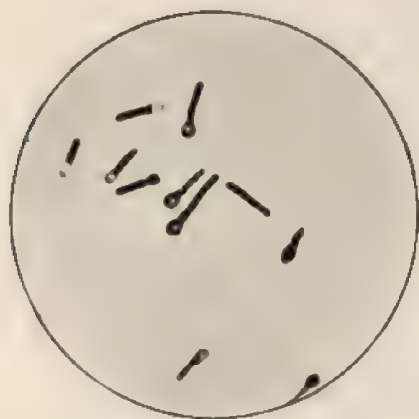
phagocytosis prevented. The associated organisms in the wound probably effect this, and do not act by producing a condition of anaërobiosis as has been suggested. The researches of Ransom and Meyer have shown that the tetanus toxin is mainly absorbed by the nerve trunks. (See also p. 147.)

Toxins. -Brieger, from impure cultures of the tetanus bacillus, obtained two basic bodies which he termed tetanine and tetano-toxin, the former producing tetanic symptoms in mice, and the latter tremor, paralysis, and finally convulsions. Brieger also isolated tetanine from the amputated limb of a tetanic patient. Brieger and Frankel obtained a tox-albumin from bouillon cultures which induced tetanus in guinea-pigs. Brieger and Cohn subsequently investigated the tetanus poison obtained by precipitating veal-broth cultures with ammonium sulphate added to saturation. The poison formed a flocculent precipitate which floated on the surface; it was purified by redissolving, precipitating the protein with basic lead acetate, and removing other soluble impurities by dialysis. So prepared, the tetanus poison forms yellow flakes, odourless, and soluble in water, but not giving the Millon and xanthoproteic reactions. It is not precipitated by most metallic salts, and is not carried down by Roux and Yersin's method of precipitation with calcium phosphate. It contains no phosphorus and only traces of sulphur. Of the most active preparation 0·00000005 gram killed a mouse. Brieger and Boer also obtained the tetanus toxin by the same method as that used for the separation of diphtheria toxin (p. 254).

In a case of tetanus examined by Sidney Martin,¹ an albumose, chiefly deutero-albumose, was extracted from the blood. Injected into an animal, it produced depression of temperature, followed by progressive wasting, but no spasm or paralysis.

¹ *Brit. Med. Journ.* 1892, i. p. 756.

PLATE XVI.



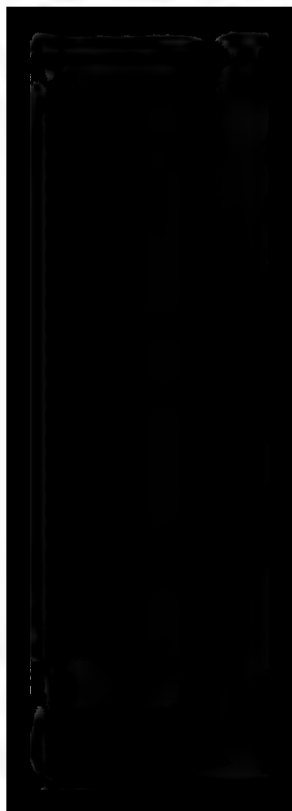
a. BACILLUS TETANI.

COVER-GLASS PREPARATION OF A PURE CULTURE. $\times 1500$.



b. BACILLUS WELCHII.

COVER-GLASS PREPARATION OF A MILK CULTURE. $\times 1000$.



Tetanus toxin has an especial affinity for nerve cells. If injected into the blood stream it is removed and fixed by the cells of the central nervous system within a few seconds. If tetanus toxin be triturated with the fresh brain of a guinea-pig in a mortar, the resulting emulsion is found to be non-toxic if injected into an animal, the toxin having entered into combination with, or being anchored to, the nerve-tissues by means of its haptophore groups. In addition to the tetanizing substance, tetanospasmin, tetanus toxin contains a hæmolysin, tetanolysin. By treatment with carbon disulphide, tetanus toxin becomes practically non-toxic, though it still retains its power of immunizing on inoculation and of combining with antitoxin—that is to say, bodies are formed analogous to the toxoids of diphtheria toxin. Injected in small doses into an animal, the muscles near the seat of inoculation are usually first affected with tonic spasm (fig. 48).

Fermi and Pernossi¹ found that agar cultures of tetanus were the most toxic—more so than gelatin and bouillon ones. According to their investigations, the tetanus poison is destroyed in an hour at 55° C. in the presence of water; it is also destroyed after exposure to direct sunlight for from eight to ten hours, and does not seem to be an enzyme.

Antitoxin.—If an animal is cautiously injected with tetanus toxin, commencing the treatment with a weakened toxin, and increasing the dose very gradually, a high degree of immunity is ultimately obtained, and the blood serum acquires marked antitoxic properties. The toxin is obtained by growing the tetanus bacillus in bouillon in an atmosphere of hydrogen for about three weeks, and filtering through porous porcelain. To obtain an active serum, treatment has to be prolonged, a horse immunized by the writer² requiring six months. The antitoxic serum

¹ *Centralt. f. Bakt.* (1^{re} Abt.) xv., 1894, p. 303.

² *Brit. Med. Journ.* 1895, i. p. 464.

so obtained is by far the most active with which we are acquainted, and is now recognized as the proper remedy to use in cases of tetanus in man. As regards the value of the antitoxic treatment of tetanus, it cannot be said to be so successful as that of diphtheria, and for this reason: in diphtheria, in a large proportion of the cases, a local manifestation is present to aid diagnosis before any serious absorption of the toxin has taken place, whereas in tetanus the disease is only recognizable by the symptoms induced



FIG. 48.—GUINEA-PIG INOCULATED WITH A SMALL DOSE OF TETANUS TOXIN, SHOWING PARALYTIC CONDITION OF RIGHT HIND LEG DUE TO SPASM.

by such absorption. Nevertheless, it can hardly be doubted that it is our duty to employ the remedy not only in the fully developed disease, but also in certain cases as a prophylactic. As the toxin is at once fixed by the nerve-tissue, the antitoxin should be injected into the central nervous system in order to obtain immediate action.

CLINICAL EXAMINATION.

The symptoms of tetanus are usually so obvious that a bacteriological examination is not needed to establish the diagnosis, and unless there is an evident wound it will be difficult, if not impossible, to detect the tetanus bacillus.

1. Prepare several cover-glass specimens of the pus or discharge, and stain by Gram's method. Examine microscopically, looking for the spore-bearing rods or 'drum-sticks.'

2. If 'drum-sticks' be found, an attempt may be made to isolate the bacillus by making anaerobic plate cultivations from the discharge, after heating it in capillary pipettes to 80° C. for half an hour.

3. Inoculate two guinea-pigs with the discharge. If they die with tetanic symptoms, treat the pus at the seat of inoculation as in 2.

MALIGNANT OEDEMA.

Malignant oedema is met with in man in connexion with wounds soiled with septic matter, compound fractures, contused and lacerated wounds, &c. Usually there is a putrefactive and oedematous condition of the tissues with subcutaneous emphysema. Animals also occasionally suffer from the disease, which can be produced artificially by inoculation with dust, dust from straw, the upper layers of garden earth, and decomposing animal and vegetable matter.

If a guinea-pig be inoculated subcutaneously with a little garden earth, it will very likely die in forty-eight hours. Post mortem, the subcutaneous tissues around the seat of inoculation will be found to be oedematous and blood-stained, with more or less development of gas. The internal organs are only slightly altered, but the spleen may be somewhat enlarged. The juice from the seat of inoculation will be found to contain a mixture of organisms, but in the blood and organs few will be found. Under the capsule of the spleen, however, long slender rods may be seen; these are the bacilli of malignant oedema.

Morphology.—The bacillus of malignant oedema is a long and slender rod, several of which may be united into a thread. It is motile, possesses several flagella, and is readily stained by the ordinary anilin dyes, but not by Gram's method. It spores freely at temperatures above 20° C., the spores being large and central.

Cultural Characters. - The bacillus of malignant œdema is a strictly anaërobic organism, but grows freely when oxygen is excluded. In a deep stab in glucose-agar it forms a thick line of growth in the needle track, with irregular outline and greyish-white in colour. There is profuse development of gas, accompanied by a foul odour, and attended with rupture of the medium into several portions.

The bacillus of malignant œdema is an organism which has to be distinguished from anthrax, and there should be no difficulty in doing this. Post mortem, the spleen is rarely found much enlarged in malignant œdema, the organism is not very abundant, is almost entirely absent from the blood, and is only found under the capsule of the spleen, not at its centre. If, however, several hours have elapsed since death occurred, the organism may have wandered into the blood and the centre of the spleen. The bacillus of malignant œdema is motile, the anthrax bacillus non-motile; the former occurs as a long slender filament, which on staining is seen to consist of two or three long segments; it does not stain by Gram's method (except by Claudius's modification), and is strictly anaërobic. Access of oxygen may render the malignant œdema bacillus non-motile.

BACILLUS WELCH.¹

Probable Synonyms. *B. aerogenes capsulatus* (Welch and Nuttall), *Granulo-Bacillus saccharo-butylicus immobilis liquefaciens* (Grassberger and Schattenfroh), *B. enteritidis sporogenes* (Klein), *B. perfringens* (Veillon and Zuber), Gas-phlegmon bacillus (Fränkel), Bacillus of acute rheumatism (Achulme).

This organism was originally described by Welch and Nuttall under the name *B. aerogenes capsulatus*, and occurs in conditions accompanied by much development of

¹ See Welch and Nuttall, *Bull. Johns Hopkins Hosp.* iii., 1892, p. 81; Welch, 'Shattuck Lecture,' *ibid.* xi., 1900, p. 185; Dunham, *ibid.* viii., 1897, p. 68; Welch and Flexner, *Journ. Exper. Med.* i., 1896, p. 5; Herter, *Bacterial Infections of the Digestive Tract*, 1907; Kamen, *Centr. f. Bakt. Orig.* xxxv., 1904, pp. 554, 686; and *Archiv f. Hyg.* liii., 1905, p. 128.

gas in the tissues, as in cases which might be described either as phlegmonous erysipelas or gangrene with subcutaneous emphysema, especially after injuries. It is also met with occasionally in perforative peritonitis and in various septicæmic and pyæmic conditions, in the puerperal state,¹ complicated stricture, &c.

The *B. Welchii* is widely distributed, and has been cultivated from the soil, dust, and contents of the intestine. Gas-bubbles found in the blood and internal organs ('foamy organs') at an autopsy seem generally to be due to this organism, but may occasionally perhaps be caused by other putrefactive bacteria.

Morphology.—The *B. Welchii* is a non-motile, sporing, anthrax-like bacillus, variable in size, being 3 to 6 μ in length (Plate XVI., *b*). It occurs singly, in short chains, or in clumps, and occasionally in long threads. It stains well with the ordinary anilin dyes and also by Gram's method. A capsule is often present, but spores are only formed in blood-serum cultures.

Cultural Characters.—The *B. Welchii* grows well on all the ordinary culture media, slowly at 20° C., rapidly at blood-heat, but is strictly anaërobic. It forms greyish-white colonies on agar, and gelatin is liquefied. In glucose-broth it produces at first a diffuse cloudiness, but later the fluid becomes clear and a whitish viscid sediment settles. Milk is coagulated, the casein forming a thick, stringy, honeycombed mass on the surface of a clear watery whey. On potato the growth is almost invisible. There is abundant formation of gas in culture media, the gas both in dextrose media and in milk, according to Theobald Smith, consisting of hydrogen and carbon dioxide in the ratio 2 : 1 or 3 : 2.

Pathogenicity.—The *B. Welchii* is pathogenic for guinea-pigs and mice, but slightly so for rabbits. The whey of a milk culture in quantities of 0.5–2 c.c. per

¹ See Little, *Bull. Johns Hopkins Hosp.* xvi., 1905, p. 136.

100 grams of body weight produces death of a guinea-pig within forty-eight hours. Post mortem, if injected subcutaneously, the hair strips readily from the skin, which may be green and gangrenous, the subcutaneous tissue may also be green and gangrenous, or more or less digested, so that the skin hangs loose, and the sac formed contains gas and exudation, sometimes scanty, sometimes abundant, thin and sanguinolent, and containing numbers of bacilli. If the post mortem be delayed, or if the heart-blood be taken up into tubes, and these sealed and incubated, many of the bacilli will be found to be sporing. Pigeons, by intramuscular inoculation, are also susceptible. Injected intravenously into a rabbit, the animal killed immediately and the carcass incubated at 37° C. for twenty-four hours and examined, there is an abundant formation of gas, particularly in the liver, which is riddled with gas-bubbles. This is a very characteristic test (Welch-Nuttall test).

The *B. Chaunvi* also produces this 'foaming' condition of organs when similarly treated, but spores freely, whereas the *B. Welchii* does not spore under such conditions. Monkeys fed with considerable numbers of *B. Welchii* are unaffected. In the human intestine the organism is almost absent or scanty in nurslings and children, but becomes more and more abundant as age advances. It is probable that it is capable of producing necrotic changes in the intestinal mucous membrane. Different strains seem to vary much in virulence.

Products and Toxins. The gas production has already been mentioned. Butyric and allied acids are freely formed, but lactic acid is scanty. Indole may or may not be produced. Hemolytic substances can be readily detected in blood-bouillon cultures, and the organism is abundant in the intestine in some cases of primary anæmia and possibly may have some relation to the condition. In some cases of infection the blood serum agglutinates the organism.

Under the name *B. enteritidis sporogenes*, Klein¹ isolated a bacillus similar to the *B. Welchii* from the evacuations of, and from milk consumed by, patients suffering from an epidemic diarrhoea which occurred in St. Bartholomew's Hospital; as did Andrewes,² from cases of diarrhoea admitted into the same hospital. Klein believed this organism to be the cause of the diarrhoea, and stated that it could not be found in the intestinal evacuations of healthy individuals. Klein also found it in water sewage, manure, and milk. The writer, however, showed that it could generally be found in the normal dejects, also in road and laboratory dust, and frequently in milk, and the opinion he formed was that it was probably a ubiquitous organism and had little to do with the diarrhoea.³ Glynn also found the organism to be very widely distributed and fed guinea-pigs with, and himself ingested, cultures without result.⁴

The *B. enteritidis sporogenes* in its morphology, staining reaction, and cultural characters is almost if not quite identical with the preceding organism, the *B. Welchii* or *B. aerogenes capsulatus* of Welch. The only point of difference between them is that the former, according to Klein, is motile and flagellated, while the latter, according to Welch, is non-motile and non-flagellated. Spores are only formed in serum or gelatin, not on agar. It is abundantly present in sewage and sewage contaminated water (see Chapter XXI.). The *Clostridium butyricum* of Batkin, an energetic butyric-acid-forming, anaerobic bacillus (p. 407), produces changes in milk similar to those of the *B. Welchii*, but is non-pathogenic.

CLINICAL EXAMINATION (MALIGNANT ŒDEMA AND *B. Welchii*).

The character of the wound and discharge will probably give some indication of the existence of infection with malignant œdema or with *B. Welchii*. The tissues are softened, œdematous, and discoloured, and soaked with a foul-smelling, sanguineous fluid, which may be frothy from the development of gas. Other bacilli will probably be present.

¹ *Rep. Med. Off. Loc. Gov. Board*, 1895-96, p. 197; *ibid.* 1897-98, p. 225.

² *Ibid.* for 1896-97, p. 225.

³ *Trans. Jenner Inst. Prev. Med.* ii., 1899, p. 70.

⁴ *Thompson Yates Lab. Rep.* iii. Pt. ii. 1901, p. 131.

1. Make cover-glass specimens from the discharge. Stain some with Löffler's blue, and others by Gram's method. Examine microscopically, and look for bacilli of the forms described. The malignant oedema does not stain, the *B. Welchii* stains, by Gram.

2. Inoculate two guinea-pigs subcutaneously with the discharge or with portions of the tissues. If the animals die, look for the characteristic organism.

3. An attempt may be made to isolate the bacillus by anaerobic cultures and plate cultivations.

BACILLUS CADAVERIS SPOROGENES.

This is another organism isolated by Klein,¹ and has to be distinguished from the *B. Welchii*. The two organisms are morphologically very similar and both stain by Gram's method, but the *B. cadaveris sporogenes* does not produce the typical changes in milk. In a culture two or three days old the milk below the cream layer commences to clear, and later this change proceeds rapidly, so that at the end of a week three layers are apparent, an upper of unchanged cream, a middle yellowish and watery, and a lower of precipitated casein. Its colonies on agar are also different, sending out ramifying, anastomosing threads from their margins, and it spores freely on agar in two to three days.

BLACK QUARTER.

Syn. Black Leg, Quarter Evil, Symptomatic Anthrax, Rauschbrand.

Black Quarter is a disease affecting sheep and oxen, and is unknown in man. The names black quarter, black leg, and quarter evil are derived from the dark discoloration of the muscles of the leg and flanks or quarters of the affected animals. When the muscles are cut into, a sanguineous discharge exudes, and in this are found slender bacilli, some of which are swollen or club-shaped from the presence of spores. The muscles are dark, slightly crepitant owing to the presence of gas, and have a rancid odour.

¹ *Centr. f. Bakt.* (1^{re} Abt.), xxv. p. 278.

The organism, the *B. (Clostridium) Chauvæi*, is a slender rod never forming long threads, is strictly anaerobic and motile, but loses its motility in the presence of oxygen. Some of the rods are cylindrical throughout, others form slender spindles, others are oval or lemon-shaped. It stains with the ordinary anilin dyes but not by Gram's method (except by Claudius's modification). Occasionally in the tissues it seems to stain by Gram. It forms endogenous spores, the spore-bearing rods being enlarged or club-shaped, and therefore should be termed a 'clostridium.'

It can be grown in deep stabs in gelatin and agar. Gelatin is rapidly liquefied. In glucose-agar it forms a thick, irregular, greyish growth, with much development of foul-smelling gas. The guinea-pig is susceptible if inoculated subcutaneously or into the muscles, the bacilli being found at the seat of inoculation, but not in the blood or internal organs. Artificial immunity can be induced in various ways: by bacilli attenuated by heat or by successive cultivations, or by heating the dried muscle to 85° to 90° C. for six hours (Kitt), also by inoculating the susceptible animal at the tip of the tail. Hanna,¹ by growing the organism in a mixture of blood plasma and broth, obtained toxins which, by careful injection, conferred immunity on rabbits, the animals after injection yielding an antitoxic serum.

Hamilton has described specific anaerobic bacilli in braxy, louping-ill, and other diseases of sheep and deer.²

CLOSTRIDIUM BUTYRICUM.

An anaerobic organism occurring in milk in which it produces a marked butyric acid fermentation with changes like those of the *B. Welchii*. It forms short rods, and also long ones 8 to 10 μ in length, and filaments are met with. Spore formation takes place freely in enlarged segments. It forms a whitish growth on agar, and gelatin is rapidly liquefied, a scum forming on the surface. It is non-pathogenic (p. 405).

¹ *Journ. Path. and Bact.* iv., 1897, p. 388.

² *Rep. Louping-ill and Braxy Com. Board of Agriculture and Fisheries*, 1906.

MANUAL OF BACTERIOLOGY

CHAPTER XIV.

CHOLERA—SPIRILLUM METCHNIKOWI—SPIRILLUM OF
SKLER AND PRIOR—SPIRILLUM TYROGENUM—SPIRILLUM
BRUM.

ASIATIC CHOLERA.

The bacteriological study of Asiatic cholera may be said
to date from the researches of Koch, who in 1884 was sent
by the German Government to investigate the disease in
Egypt and India. He described an organism present in
the intestine and in the dejecta which he believed to be the
causative agent, and termed it the "Comma Bacillus."

It is more or less abundant in the intestine and in the alvine discharges, especially in the rice-like flakes, but is not found in the blood, organs, or tissues. In the rice-like flakes it is frequently so numerous that in a cover-glass specimen the 'commas' appear in masses crowded together and lying parallel to one another (known as the 'fish-in-stream' arrangement). It stains well with the ordinary anilin dyes, especially with dilute carbol-fuchsin, but is decolorized by Gram's method. It is actively motile, and possesses a single terminal flagellum at one end only, but there is some variation in this respect. Spores are not formed, though in old cultures Hueppe has described bodies which he believes to be arthrospores. In such cultures the bacilli lose their regular form, and swollen and distorted involution forms are seen.

Under cultivation in bouillon or in a hanging-drop specimen it is found that the organism develops into a longish spiral filament, and the commas are therefore regarded as resulting from the breaking up of a spirillum; but if the conditions of growth are very favourable multiplication may be so rapid that the curved rods or commas are alone produced, the organism dividing before it has had time to grow into a spiral. Such a curved rod is commonly known as a 'vibrio.'

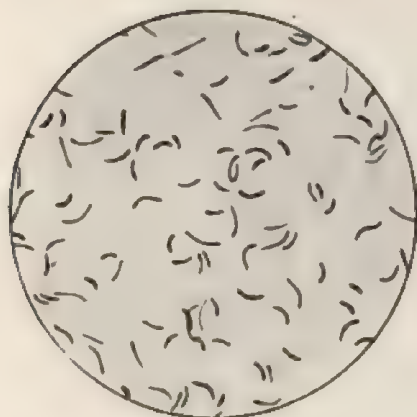
Cultural Characters and Biology.—The Koch's spirillum is aerobic and facultatively anaerobic, and grows well on the ordinary culture media from 20° to 37° C.

According to Frankland, although it grows readily in an atmosphere of hydrogen, it does not develop in one of carbonic acid gas. In gelatin plates at 22° C. small cream-coloured colonies appear in about twenty-four hours, soon accompanied by liquefaction, so that in two or three days the plate becomes pitted. Microscopically, the young colonies are rounded with irregular margins, cream-coloured, and coarsely granular. In stab-cultures development occurs

all along the stab as a whitish, opaque, punctate growth, thicker above than below. Liquefaction commences about the second day and progresses slowly; in the early stage it is confined to the surface, and looks like a little bead or air bubble (Plate XVII., *b*), but in a fortnight or so the greater part of the gelatin may be liquefied. Liquefaction varies greatly both in rate and extent in different cultures and stocks; in some old laboratory cultures it may be almost or quite absent. On surface agar a thick, moist, shining, greyish growth quickly develops with more or less crenated margins, often becoming brownish when old. On blood serum much the same growth occurs with slow liquefaction. A thin brownish layer is formed on potato at 37° C., and broth becomes turbid, and a film forms on the surface. Peptone water, or Dunham's modification of it (1 per cent. NaCl), is a good cultivating medium and becomes turbid, especially in the upper layers. In milk it multiplies rapidly without curdling; neutral litmus glucose-agar is reddened from the development of acid, but no gas is produced under cultivation. Acid, but not gas, is produced from glucose, maltose, saccharose, lactose, and starch.

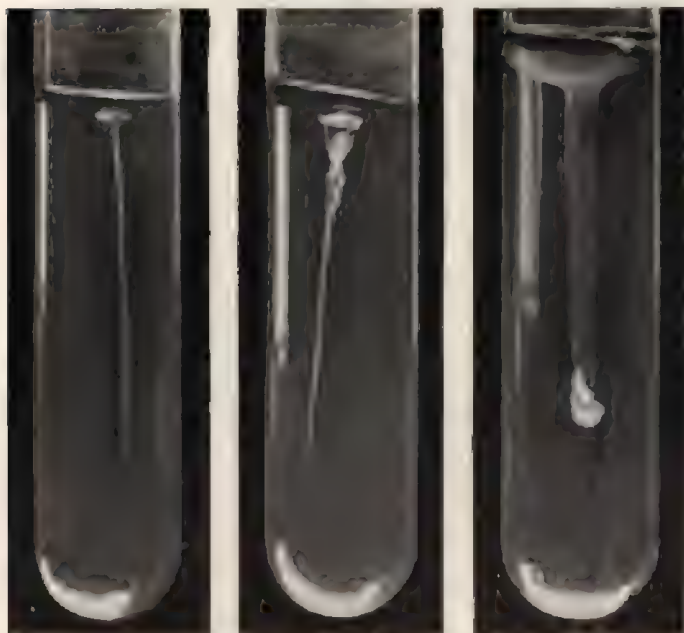
An important characteristic of the cholera spirillum is the rapid formation of indole in considerable quantity, and the reduction of nitrates to nitrites, especially in peptone water. This forms the basis of the important cholera-red reaction; a few drops of pure sulphuric or hydrochloric acid added to a peptone-water culture, eight to twelve hours old, give a pink colour, and the colour is intense when the culture is two to three days old, and of a purplish-red colour, like that of potassium permanganate. Some specimens of 'peptone' are unsuitable for the peptone water used for obtaining the reaction, apparently on account of the absence of nitrates and nitrites. The medium should be sugar free, and the addition of 0·01 per cent. potassium nitrate to it is an advantage. Some believe that two

PLATE XVII.



a. SPIRILLUM CHOLERAÆ.

COVER-GLASS PREPARATION OF A PURE CULTURE. $\times 1500$.

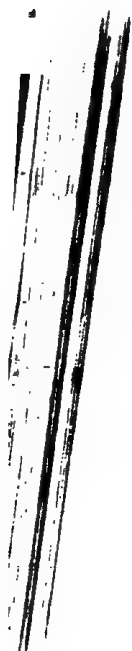


b.

c.

d.

GELATIN STAB-CULTURES, 2 DAYS OLD, OF (*b*) *SP. CHOLERAÆ*,
(*c*) *SP. METCHNIKOWI*, (*d*) *SP. FINKLERI*.



pigments are formed in the reaction, a cholera-red and the nitroso-indole pigment.¹ The reducing action of the cholera spirillum can also be shown by growing in litmus broth, which becomes decolorized (Cahen's test).

Kraus and Prantschoff² noticed that certain vibrios dissolved red-blood corpuscles, but came to the conclusion that no true cholera vibrio is hæmolytic.

The cholera spirillum retains its vitality in cultures for a month. It can multiply in water and on the surface of moist linen, but rapidly dies on complete desiccation. Its thermal death-point, according to Sternberg, is 52° C. with an exposure of four minutes; according to Kitasato, 55° C. in about ten minutes. It is readily destroyed by the ordinary antiseptics—hydrochloric acid 1 in 1800, carbolic acid 1 in 400, mercuric chloride 1 in 10,000, the time of exposure being two hours.

In some experiments by Dempster³ it was found that the comma bacillus lived for three to five days in dry soil, but only one day in an artificially dried soil, while in moist soil it lived for twenty-eight to sixty-eight days. In peat, however, it was invariably dead within twenty-four hours. In sterilized salt solution (0.75 per cent.) the comma bacilli were alive on the 159th day, and in fresh urine (sterilized) they lived fourteen days at 37° C. and twenty-nine days at 22° C.

In sterilized distilled water the cholera spirillum usually rapidly dies, within twenty-four hours as a rule. The addition of sodium chloride greatly increases the length of time it may remain alive, a survival of five or six weeks having been recorded. In ordinary sterilized potable waters it may survive many months. In unsterilized potable

¹ Wherry, Bureau of Government Laboratories, Manila, *Bulls.* 19 and 31, 1904 and 1905.

² *Wien. klin. Woch.* 1906, p. 299.

³ *Med. Chirurg. Trans.* lxxvii., 1894, p. 263.

waters its survival is greatly influenced by the presence of salts ; in some cases it dies out rapidly ; in others, especially in those containing a large proportion of salts, it may remain alive for a long time. In sterilized sewage the cholera spirillum multiplies and survives for months, in unsterilized sewage it may survive for two to four weeks (Houston).

The relation of the cholera spirillum to the disease has been a very vexed question in the past, but the outcome of the voluminous researches which have been made is to confirm Koch's work. The organism is found in all cases of cholera, and several instances of laboratory infection from cultures are on record.

None of the lower animals suffers from or contracts a disease in any way comparable to Asiatic cholera, so that the test of animal experiments cannot be applied except in the case of young suckling rabbits (see below, ' Anti-serum '). By first neutralizing the acidity of the gastric juice by an injection of sodium carbonate solution into the stomach, then diminishing peristalsis by an injection of tincture of opium into the peritoneal cavity, and finally injecting a broth culture of the cholera spirillum into the stomach, Koch succeeded in inducing in guinea-pigs a condition somewhat similar to cholera in man—namely, indisposition with falling temperature, weakness of the extremities, and death in forty-eight hours. Post mortem, the small intestine was congested and filled with a watery fluid containing large numbers of the cholera spirillum. Injected into the peritoneal cavity of mice, guinea-pigs and rabbits, it usually produces death from a general septicæmia.

Metchnikoff¹ ascribes the immunity of animals to intestinal cholera as largely due to the inhibitory action of the other organisms present in the digestive tract. In man digestive disturbances are often an important pre-

¹ *Ann. de l'Inst. Pasteur*, vii. pp. 403, 562 ; viii. pp. 257, 520.

disposing cause of an attack. The acidity of the gastric juice is also probably a means of defence (see 'Water').

That the cholera spirillum is associated with the disease seems to be beyond any doubt, and so constant is its presence in true cholera that all investigators, even those who at one time opposed Koch's views, rely on its detection for the bacteriological diagnosis. The matter, however, has become complicated owing to the detection in various natural waters of pathogenic spirilla which, although not identical with the cholera spirillum of Koch, resemble it so closely that it is difficult to classify them as anything but varieties of the cholera spirillum. In certain epidemics in India variations have also been noted in the cholera spirilla that have been isolated. Sanarelli¹ isolated from the Seine and Marne thirty-two spirilla, of which four were almost indistinguishable from cholera, except that they were only slightly pathogenic, but by passage through a series of animals their pathogenic power was much enhanced. Sanarelli believed that these were the descendants of true cholera spirilla that had gained access to the rivers during some previous epidemic of cholera. At the same time it is to be noted that vibrios may also be present in the normal intestinal tract of man and animals, and might therefore gain access to streams (Sanarelli). Dunbar similarly, from the Elbe and Rhine, &c., isolated a number of spirilla which could not be distinguished from the cholera spirillum (*Spirillum Elucers*). It was afterwards noticed that some of these under certain conditions of oxidation and temperature became phosphorescent,² but Rumpel³ has also found that cultures of the genuine cholera spirillum may exhibit phosphorescence, so this cannot be used as a differential character for the separation

¹ *Ann. de l'Inst. Pasteur*, vii. p. 693, and ix. p. 129.

² *Centr. f. Bakt.* (1^{re} Abt.) xviii., 1895, p. 424 (Kutscher).

³ *Munch. Med. Wochenschr.*, 1895, No. 3.

of non-choleraic forms. Neisser isolated a spirillum, which he termed *Vibrio Berolinensis*, which agreed with the cholera spirillum in every particular except that the colonies in a gelatin plate were invisible to the naked eye in forty-eight hours. Heider found in the Danube a spirillum, named by him the *Vibrio Danubicus*, which resembled the cholera spirillum closely, but its colonies were somewhat different, and it was more actively pathogenic to mice. Ivanoff similarly obtained a spirillum which could only be distinguished from cholera by the finer granulation of its colonies and more distinct spiral form. Lastly, there is the *Spirillum Massowah*, isolated from an epidemic of cholera at Massowah, which differs from the Koch spirillum in having two terminal flagella at each end. Cunningham has also described several spirilla differing slightly from the cholera spirillum.

Applying the Pfeiffer and agglutination tests to the spirilla in question, the following results were obtained. In the first place, each of the organisms gives a complete positive reaction to both tests with its own serum; this, of course, is only to be expected. Pfeiffer found that, using his reaction, the variety *Ivanoff* gave a positive reaction with cholera serum, and Durham found that *Ivanoff* and *Berolinensis* reacted completely with cholera serum. Conversely, positive reactions with cholera spirilla were obtained with *Massowah*, *Danubicus*, and *Elvers* sera, while *Massowah* and *Elvers* react completely to each other. From these considerations it would therefore seem probable that some of these spirilla—*Sanarelli*, *Berolinensis*, and *Ivanoff*—may be varieties of the Koch spirillum. The *Massowah* spirillum is usually considered not to be a true cholera vibrio.

Ruffer¹ in 1905 at El Tor isolated vibrios, which may

¹ *Researches on the Bacteriological Diagnosis of Cholera*. Sanitary, Maritime, and Quarantine Council of Egypt, Alexandria, 1907. (Also *Brit. Med. Journ.* 1907, i. p. 735.)

be distinguished as 'El Tor vibrios,' from the intestine of pilgrims returning from Mecca and suffering from various diseases (dysentery, diarrhoea, pneumonia, rheumatism), but among whom there had been no cholera, nor had they been in contact with cholera. These vibrios were subjected to detailed examination by the agglutination, saturation and fixation tests, and Pfeiffer's reaction with Berlin cholera immune serum, and also by the hæmolysis test. Vibrios isolated from a previous epidemic of cholera (referred to as Group 1), and other vibrios isolated from cholera and other stool (Groups 3 and 4), were also compared with the El Tor vibrios. Ruffer's results were as follows:

Group 1 (undoubted cholera vibrios).—Those which react positively to the four principal tests with cholera serum—namely, the agglutination, saturation, and fixation tests, and Pfeiffer's reaction. They do not hæmolyse, even when remaining in contact with red corpuscles for three days at the temperature of the laboratory.

Group 2. —The second group contains the vibrios agglutinated by and giving the saturation and Pfeiffer's reactions with cholera serum, but not fixing the cholera-immune body. These vibrios are strongly hæmolytic. This group consists of the El Tor vibrios only.

Group 3. The third group is formed by vibrios which are not agglutinated by immune serum, do not give the saturation or Pfeiffer's reaction, but fix the cholera immune body. These vibrios also hæmolyse, but feebly and late, often after thirty-six to forty-eight hours only.

Group 4.—The last group is formed by strongly hæmolytic vibrios not reacting at all to cholera immune serum.

Ruffer concludes that the El Tor vibrios are not genuine cholera vibrios. He says: 'The only possible classification is to group together all the vibrios reacting in the same way to all tests, separating them from those

which, under the same conditions, behave in a different way. If this method be applied to the vibrios found at El Tor, there is no difficulty in distinguishing them from the true cholera vibrios, in spite of several of the reactions of both being similar. And it follows also that the agglutination, saturation and Pfeiffer's tests are not in themselves of absolute diagnostic value for cholera vibrios.'

Neufeld and Haendel,¹ however, after a re-examination of some of these vibrios, consider that they are true cholera vibrios. The matter therefore remains undecided.

The variability of the Koch spirillum is shown by experiments of Klein² on the relation of oysters to disease. He ascertained that the cholera vibrio could retain its vitality for at least fourteen days in unsterilized sea-water, while from the interior of oysters, kept in water infected with the vibrios, it was obtained up to nine days after infection. Some of the vibrios obtained from the water and from the oysters showed, however, marked variation from the parent stock—variations in the rate of liquefaction, in the curdling of milk, in the indole reaction, &c.; and five more or less definite varieties were obtained, two of which did not respond to Pfeiffer's test, although originally derived from a genuine cholera vibrio responding to all the tests, including Pfeiffer's. In the East many cases of cholera are mixed 'vibrionic' infections; the stools may contain several varieties of vibrios, some agglutinating with cholera serum, others not; some monociliate, others multiciliate.

Toxins.—Brieger in 1887 obtained cadaverin and putrescin and two other basic bodies from cholera cultures. Brieger and Frankel isolated a tox-albumin, and Gamaleia a ferment-like body. Hueppe believes that the cholera poison is a tox-albumin found in the culture medium, but

¹ *Arbeit. a. d. Kais. Gesundheitsamte*, xxvi., 1907, p. 536.

² *Rep. Med. Off. Loc. Gov. Board* for 1896, p. 135.

that immunizing substances are derived from the bacterial cells.

Rontaler compared the chemical products of the ordinary and of the Massowah cholera spirilla, and could find little difference between them.

Wesbrook¹ investigated the toxins produced by the Koch spirillum in different media. From alkali-albumin cultures he isolated deutero-albumose and traces of proto-albumose, from cultures in eggs a mixture of protein bodies which it was impossible to separate, from asparaginate of sodium (Uschinsky's medium) a substance which gave a feeble xanthoproteic, but not the biuret reaction, and from the peritoneal exudation of inoculated guinea-pigs a substance which, although it gave a slight xanthoproteic reaction, did not appear to be either deutero- or proto-albumose. This observer also found aërobic cultures of the cholera spirillum to be much more toxic than anaërobic ones.

Pfeiffer found that cholera cultures killed with chloroform vapour contained a toxic substance fatal to guinea-pigs in small doses, with extreme collapse. He believed the substance to be an integral part of the bacterial cells.

Metchnikoff² and his co-workers have demonstrated the existence of a soluble cholera-poison in a very ingenious manner. Collodion sacs of 2 c.c. to 3 c.c. capacity were sterilized, filled with peptone solution, inoculated with the cholera spirillum, and closed. The closed sac was then introduced into the peritoneal cavity of a guinea-pig, which died in three or four days from the effects of the soluble toxins dialysing through the walls of the sac (see next page).

Macfadyen obtained a highly toxic endotoxin by triturating cholera cultures with liquid air.³

¹ *Ann. de l'Inst. Pasteur*, viii., 1894, p. 318; *Journ. of Path. and Bact.* iv., 1896, p. 1.

² *Ann. de l'Inst. Pasteur*, x., 1896, p. 257.

³ *Lancet*, 1906, ii. p. 494.

Anti-Serum.—By growing the cholera spirillum in a shallow layer with free access of oxygen in a peptone-gelatine-salt medium, Metchnikoff and his co-workers obtained a toxic fluid after three or four days' growth. During incubation the fluid becomes concentrated to about one-eighth by evaporation. After filtration, 0.25 c.c. killed a 300-gram guinea-pig in eighteen hours. Goats having been inoculated with increasing doses of this toxin, commencing with 10 c.c. and reaching 200 c.c. in six months, become immunized and yield an antitoxic serum, 1 c.c. of which will neutralize four times the lethal dose of toxin. Metchnikoff had previously found that young suckling rabbits suffer from an intestinal cholera when fed with cultures, so that the effect of the cholera antitoxin in preventing intestinal cholera could be tested on these animals. Experiment showed that of the treated rabbits 51 per cent. survived, of the untreated only 19 per cent.

Animals may be inoculated with dead and living cultures and an immune serum so prepared, but no practical value has yet attended the use of anti-sera in the treatment of cholera. Macfadyen immunized a goat with cholera-cell juice and obtained a serum of which $\frac{1}{800}$ c.c. protected a guinea-pig against three lethal doses of cholera culture.

The blood serum of an animal immunized by injections of the cholera spirillum gives a typical agglutination reaction with recent cultures of the organism. The reaction can also be obtained with the blood serum of cholera patients, sometimes as early as the first day of the disease, but it is probably of little use for diagnostic purposes, as the course of the disease is generally so rapid.

Vaccine.—Ferran in 1885 first prepared a vaccine by making cultures (mixed) in broth from cholera stools and injecting 0.3–0.5 c.c. subcutaneously, but the reports of commissions sent to investigate the method were unfavourable.

Haffkine subsequently prepared a vaccine against cholera from cultures of the Koch spirillum, which seems to be efficacious in preventing the disease. For example, a number of labourers were inoculated during an epidemic, and among the inoculated the mortality was only 2·25, whereas among the uninoculated it was nearly 19 per cent. In another instance, among 654 uninoculated there were seventy-one deaths, a mortality of 10·86 per cent., while among 402 inoculated there were only twelve deaths, a mortality of 2·99 per cent., and a reduction in mortality of 72·47 per cent.¹

Besredka,² by making a mixture of cholera culture and cholera-immune serum, allowing this to stand for twelve hours, heating to 56° C. for one hour and then injecting subcutaneously, claims that an immediate and lasting (six months) immunity may be produced.

Strong³ prepares a vaccine from autolysed cultures. The cholera vibrio is grown on surface agar for twenty-four hours at 37° C.; the growth is then washed off with sterile water, the suspension is kept at 60° C. for twenty-four hours, and then at 37° C. for two to five days, and is finally filtered through a porcelain filter.

Haffkine makes use of two vaccines, which are prepared as follows:⁴

The first vaccine is prepared from attenuated cultures of the cholera spirillum. The ordinary laboratory cultures are usually considerably attenuated, but to be sure that they are sufficiently so they are grown for several generations on surface agar at 38° C. in tubes through which a current of moist air is continuously passed. Such a culture causes only a local oedema

¹ *Rep. on Inoc. against Cholera and Typhoid Fever in the East Indies*, 1900.

² *Ann. de l'Inst. Pasteur*, 1902, p. 918.

³ Bureau of Gov. Laboratories, Manila, *Bull.* No. 16, 1904 (Bibliog.).

⁴ *Brit. Med. Journ.* 1893, i. p. 227 (Wright and Bruce).

instead of necrosis when injected into the subcutaneous tissue of a guinea-pig.

The second, or strong vaccine, is prepared from cholera cultures, the virulence of which has been artificially increased by growing in the peritoneal cavity of guinea-pigs. This is done by first of all preparing 'standard' cultures from any ordinary culture of cholera. Test-tubes measuring 15 cm. in length are employed; of the 15 cm., 10 cm. are occupied by the sloping surface of ordinary nutrient agar. The whole surface of the nutrient medium is inoculated and the inoculated tubes are incubated at 35° C. for twenty-four hours. The whole growth from the surface of the agar is then scraped off with a sterilized platinum needle of stout wire and made into an emulsion with about 8 c.c. of sterile broth. A guinea-pig (800-400 grm.) is etherized, a small patch of hair on the abdomen cut short, and a spot cauterized with a hot iron to sterilize it. The emulsion of cholera bacilli is then drawn up into a sterile syringe or glass pipette and injected into the abdominal cavity through the cauterized area. Two guinea-pigs should be injected at the same time, using for each one a standard cholera culture. The guinea-pigs so treated will die within twenty-four hours.

The peritoneal cavity of the dead animal is opened aseptically, and with a sterile glass pipette the peritoneal fluid is sucked up from the iliac fossæ. The whole of the peritoneal fluid from one guinea-pig is introduced into a sterile test-tube which is well plugged with cotton-wool and placed in the oblique position (for aeration) in the incubator at 35° C. for about ten hours. This is to allow of the proliferation of the cholera bacilli. After this treatment the fluid is injected into a second guinea-pig, the size of which, however, has to be taken into account. If the peritoneal fluid in the first guinea-pig be abundant, it will contain comparatively few cholera bacilli, and a smaller animal should be chosen, but if it be scanty the comma bacilli will be numerous, and a larger animal may be used. After twenty to thirty passages through guinea-pigs the virus will have attained its maximum virulence, which is known by the fact that further passages do not shorten the period which elapses between inoculation and death.

The 'exalted' cholera cultures do not retain their maximum virulence for longer than ten days, and have again to be passed through guinea-pigs (three or four).

In order to prepare the vaccines a 'standard' agar tube is inoculated over its whole surface and incubated at 35° C. for twenty-four hours. Three or four cubic centimetres of sterile broth are introduced into the tube and an emulsion is made with the whole of the growth. The emulsion is measured by drawing it up into a sterile syringe, the contents of which are then introduced into another sterile glass and made up to a volume of 8 c.c. by the addition of more sterile broth. One cubic centimetre of this emulsion constitutes the dose for vaccination. Carbolyzed vaccines may be prepared by using a $\frac{1}{2}$ per cent. solution of carbolic acid (sterilized by boiling) for making the emulsions and diluting them to 6 c.c., and not 8 c.c., as in the uncarbolyzed. The carbolyzed vaccines may be preserved for some time in sealed tubes.

The dose of vaccine (1 c.c.) is injected hypodermically into the flank, the second or strong vaccine being injected three to five days after the first or weak one.

CLINICAL DIAGNOSIS.

Some of the rice-like flakes should be picked out of the stool and well rinsed in sterile salt solution.

1. From one of the whitish, slimy, rice-like flakes in the evacuations or the intestine cover-glass specimens are prepared, stained in Löffler's blue, washed, dried, and mounted. If on examination large numbers of curved rods lying in groups parallel to one another are observed, the diagnosis of Asiatic cholera may be made with some degree of certainty. Koch states that this is so in quite half the cases, especially the acute ones. (Single, or a few, vibrios are of no diagnostic significance; they may occur in normal and diarrhoea stools.)

2. A set of gelatin plates and a set of agar plates should be prepared from an emulsion of rice-like flakes. In the case of the agar plates they should be prepared by pouring the melted agar into the Petri dishes, allowing it to set, and then smearing the flake over the surface. The plates are incubated at 22° C. and 37° C. respectively. In the gelatin plates the characteristi

colonies of the cholera vibrios should be recognizable in about twenty-four hours, in the agar plates in from twelve to sixteen hours. The likely colonies should be examined microscopically and peptone-water and other cultures prepared from them.

3. With other rice-like flakes several peptone-water cultures should be prepared and incubated at 37° C. This is best done in small Erlenmeyer flasks containing a shallow layer (1-2 cm. deep) of Dunham's peptone water, without wool plugs, but capped with a piece of sterile filter-paper. In eight to ten hours the upper layers of the fluid should be examined microscopically for the presence of commas, and gelatin and agar plates and subcultures in peptone water also made by inoculating from the surface layer of fluid. The peptone-water culture may then be tested for the presence of indole by carefully adding a few drops of pure concentrated sulphuric acid. In cases of Asiatic cholera the indole reaction can be obtained as early as eight hours after inoculation.

4. To vibrios that have been isolated, the agglutination, saturation, and fixation tests and Pfeiffer's reaction should be applied, a high-grade authentic cholera-immune serum being used. The hemolysis test should also be applied (p. 165).

5. If the case has lasted any time the agglutination reaction may be applied, testing the patient's serum on a known strain of cholera vibrio, but this is of doubtful value.

SPHIRILLUM METCHNIKOWI.

Isolated by Gamaleia¹ from the intestinal contents of chickens dead of an infectious gastro-enteritis which occurred in certain parts of Russia. The disease, although resembling chicken cholera in some respects, is quite distinct from the latter. This spirillum forms curved rods and spiral filaments, generally slightly shorter, thicker, and more curved than the Koch spirillum. It is decolorized by Gram's method, and is best stained with weak carbol-fuchsin. It is readily cultivated and is aerobic and facultatively anaerobic. In gelatin plates it forms small

¹ *Ann. de l'Inst. Pasteur*, ii., 1888, p. 482.

whitish colonies, visible within twenty hours, which grow more rapidly than the cholera vibrio, and in two or three days produce marked areas of liquefaction. In a stab-culture in gelatin a whitish granular growth occurs along the line of puncture with liquefaction, much like that of the Koch spirillum, but the rate of growth and the liquefaction are more rapid (Plate XVII., c). Grown in eggs by Hueppe's method typical appearances are produced. After ten days the white becomes transformed into a yellowish limpid liquid, while the yellow, though retaining its form and consistence, is quite black. On surface agar a thick cream-coloured layer develops, on potato the growth is brownish, and milk is coagulated. It grows freely in broth and peptone water, the fluid becoming uniformly turbid, and a slight film forming on the surface, and these cultures give a marked indole reaction on the addition of sulphuric acid alone, in this respect resembling the Koch spirillum. The *S. Metchnikowi* is pathogenic to chickens, pigeons, and guinea-pigs, but not to rabbits or mice except in large doses. It is, however, more pathogenic to guinea-pigs than the cholera vibrio, pigeons are killed by intramuscular inoculation, and fowls are susceptible to feeding, whereas the cholera vibrio is not fatal to pigeons and fowls under these conditions. It is not agglutinated with cholera-immune serum. Abbott¹ isolated a pathogenic spirillum from the Schuylkill River, Philadelphia, which resembles the *S. Metchnikowi* closely, and is probably identical with it.

SPIRILLUM FINKLERI (OF FINKLER AND PRIOR).

Isolated from the stools in certain cases of cholera nostras, but its aetiological significance is doubtful. It occurs as short, thickish, curved or straight rods, and sometimes as spiral filaments. It is aerobic and facultatively anaerobic, does not form spores, and does not stain by

¹ *Journ. of Exper. Med.* i., 1896, p. 419.

Gram's method. In a gelatin stab-culture a yellowish growth forms with rapid liquefaction (Plate XVII., *d*). On agar a thick, slightly brownish, moist layer develops. Serum is rapidly liquefied. On potato a slimy brownish growth occurs even at room temperature. It grows in broth and peptone water, producing a general turbidity. It does not as a rule give the indole reaction with sulphuric acid alone, but the ordinary laboratory cultures after three to four days' growth occasionally give a slight reaction. It is stated to be pathogenic to guinea-pigs by intraperitoneal inoculation.

SPIRILLUM TYROGENUM.

Obtained by Deneke from old cheese, and frequently spoken of as Deneke's spirillum. It forms curved rods and spiral filaments somewhat closely resembling the Koch spirillum. It grows well on the ordinary culture media at room temperature, but development is usually slight or absent at 37° C. In a gelatin stab-culture a yellowish growth occurs with liquefaction, which is much more rapid than that of the Koch spirillum, but less so than that of the Finkler-Prior spirillum. On agar a thinnish, brownish, somewhat membranous and coherent layer slowly develops at room temperature. On potato a yellowish growth occurs. It is stated to be slightly pathogenic to guinea-pigs by intraperitoneal inoculation.

SPIRILLUM RUBRUM.

A chromogenic spirillum obtained by Koch from the putrefying tissues of a mouse. In a gelatin stab-culture a dark red growth slowly develops along the line of puncture without liquefaction; at the surface, however, the growth is colourless. In broth at 37° C. it grows freely, producing a general turbidity with a red deposit at the bottom of the tube; there is no film formation. In such a broth culture large numbers of typical spirillar filaments can be seen, which are thin and delicate, of varying length, and actively motile. It is non-pathogenic.

Vibrios are common in the mouth, and may be met with in the discharge of septic ulcers.

CHAPTER XV.

STREPTOTHRIX INFECTIONS — ACTINOMYCOSIS — MYCETOMA —
LEPTOTHRIX BUCCALIS — CLADOTHRIX DICHOTOMA — MYCOSIS
TONSILLARIS.

STREPTOTHRIX INFECTIONS.

The Streptotricheæ are a group of thread-forming organisms showing true, but not dichotomous, branching. Their exact position in the botanical scale is uncertain; by some they are considered to belong to the higher Schizomycetes, forming a connecting link between these and the Hyphomycetes, but the general view is rather to place them among the latter.

The Streptotricheæ form a filamentous network, or mycelium, the individual threads of which show branching, while their terminal portions undergo segmentation, with the formation of rounded bodies regarded as spores. The mycelial network, unless old, stains by Gram's method, and occasionally possesses 'acid-fast' properties.¹

Pathogenic streptothrix forms are not uncommon, the best known being those causing actinomycosis and the white variety of mycetoma. Others affect the lower animals,² and a few affect man.³ Doubtless cases of streptothrix infection in man may occasionally be missed, as the clinical characters are those of tuberculosis. (For a monograph on

¹ See Birt and Leishman, *Journ. of Hyg.* ii. Pt. ii. 1902.

² See Dean, *Trans. Path. Soc. Lond.* li. Pt. i. p. 26 (Bibliog.), and Foulerton, *Journ. Comp. Path. and Bact.* xiv., 1901, Pt. i. p. 45.

³ Flexner, *Journ. Exp. Med.* iii., 1898, Nos. 4 and 5.

MANUAL OF BACTERIOLOGY

Leptotrichæ see Foulerton and Price-Jones, *Trans. Soc. Lond.* liii. Pt. i. 1902, p. 56.)

ACTINOMYCOSIS.¹

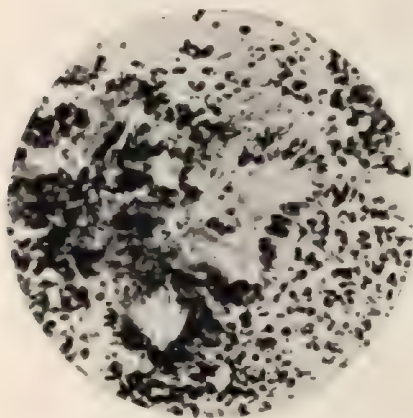
In man, actinomycosis in its clinical history and pathological lesions closely resembles tuberculosis, and doubtless of it have been, and are, frequently confounded with scrofula.

In cattle, actinomycosis has long been known, but its pathology was involved in considerable doubt until the researches of Bollinger in 1876. It forms tumours affecting the tongue, jaw, face, and throat, and is described under such varied names as wen, scrofula, osteo-sarcoma, cancer, wooden tongue, &c.

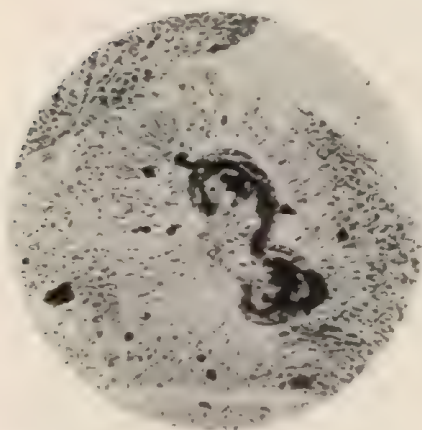
The tumours after a time break down and discharge, the tongue often protrudes from the mouth, the saliva and the animal becomes much emaciated.

On cutting into a 'wooden tongue' or wen, a grating sep-

PLATE XVIII.



a. ACTINOMYCOSIS BOVIS.
SECTION OF TONGUE. GRAM. $\times 350$.



b. MYCETOMA. SECTION OF TISSUE, WHITE VARIETY.
GRAM. $\times 350$.



somewhat soft in consistence and on slight pressure flatten out. Examining with a high power, these granules are found to contain round, ovoid, or reniform bodies which have a rosette-like appearance, a more or less structureless centre with club-shaped bodies radially arranged around the periphery (Plate XVIII, a). These peculiar structures are the cause of the disease, and are the form assumed in the animal body by an organism belonging to the streptothrix group termed the *Actinomyces*, or *Streptothrix bovis*, or, from its appearance, the ray fungus.

Sections of the diseased tissues show the structure of the organism still better. Gram's method usually gives good results, and it will generally be found that the following appearances can be observed: Surrounded by the round cells are the reniform or ovoid bodies, situated at the periphery of which are radially arranged, club-shaped structures deeply stained with the gentian violet, while the central portion is unstained and structureless, or contains granular matter or calcareous particles. Various appearances may be met with in different parts of the section, according as the actinomycotic nodules are cut through their centre or periphery; when the latter is the case, the clubs are shown in transverse section and appear as cloudy packed, deeply stained dots. Sometimes, however, in addition to the clubs the centre of the rosette is occupied by numerous interlacing filaments, also stained by the gentian violet.

In man, actinomycosis is often associated with suppuration. If a little of the pus be examined it will probably contain tiny yellowish or sulphur yellow granules, which, microscopically, are found to have the same structure as the granules met with in the disease in cattle and described above—namely, rosette-like tufts of clubs, but in addition, occupying the centre of the tufts, is a mass of fine tangled filaments, the ends of which are frequently seen to be continuous with the clubs. In tensed-up specimens, or in

sections stained by Gram's method, an appearance is observed very different from that of the bovine variety, viz. tufts of interlacing filaments stained by the gentian violet, but a complete absence of purple clubs (Plate XIX., *a*). The clubs, however, are not really absent; they are present abundantly all round the periphery of the filamentous tufts, in a stunted condition it is true, and they do not usually stain by Gram's method. These clubs are very well seen in fresh specimens of the pus or in unstained sections, or by

staining with orange-rubin, or the Ehrlich-Biondi reagent (Plate XIX., *b*). The conditions in cattle and man, at first sight so very different, are thus seen to be similar, and their identity is further established by the occasional occurrence in cattle of filamentous tufts, staining by Gram's method, within the rosettes, and by the clubs in man now and then taking on the gentian-violet stain.

Cultural Characters.—The cultivation of the *Actinomyces* can be performed by collecting the pus from a case of the disease in sterilized tubes, and subsequently turning it out into a sterilized capsule and picking out the actinomycotic granules with sterilized needles, planting these on the surface of glycerin agar, and incubating at 37° C. A certain number of the tubes will probably be uncontaminated, but in others a growth of the *Micrococcus pyogenes* var. *aureus* or other pyogenic

organism, which is not unfrequently associated with the *Actinomyces*, may occur. In the uncontaminated tubes a growth begins to appear in a few days in the form of little colonies of a tough membranous consistence, some-

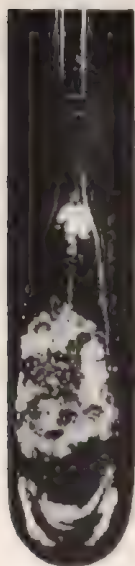
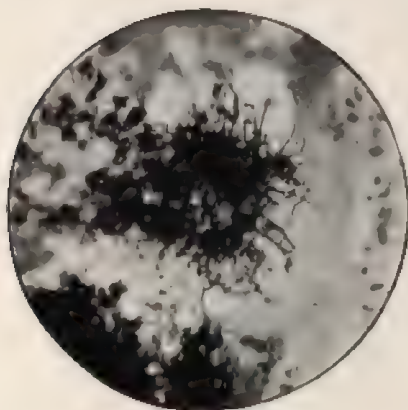


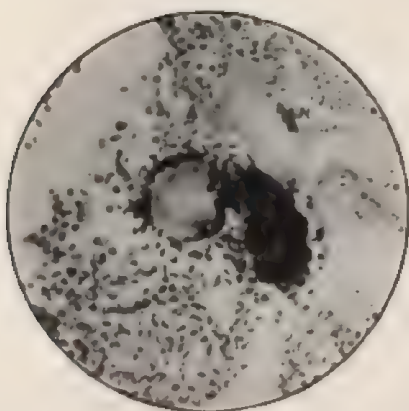
FIG. 49. — ACTINO-
MYCES. POTATO
CULTURE, THREE
MONTHS OLD.

PLATE XIX.



a. ACTINOMYCOSIS HOMINIS.

SECTION OF LIVER SHOWING A MYCELIAL MASS. GRAM. \times 500.



b. ACTINOMYCOSIS HOMINIS.

SECTION SHOWING A RING OF STUNTED CLUBS. GRAM. \times 350.

SAME MATERIAL AS FIG. a ABOVE.



what wrinkled, greyish, and shining, while the agar beneath them becomes stained brownish. The growth increases and the colonies coalesce, forming a brownish, wrinkled, membranous expansion, sticking firmly to the agar and difficult to remove or break up, while the agar becomes stained brown throughout; later on the membranous growth may become dappled with yellow as though powdered with flowers of sulphur, but occasionally remains whitish. In gelatin, little spherical feathery tufts develop and sink to the

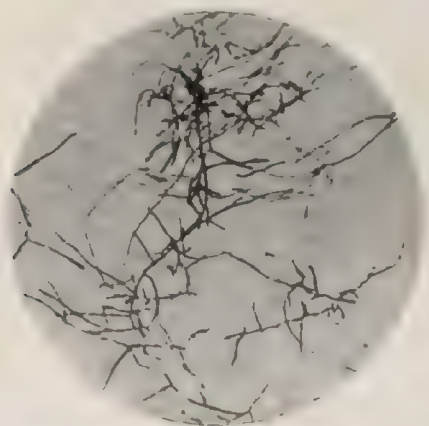


FIG. 50.—ACTINOMYCES. COVER-GLASS PREPARATION.
GRAM. $\times 750$.

bottom as liquefaction progresses. On potato a remarkable growth develops; at first brownish, it afterwards becomes almost black and is very thick or heaped up with a much wrinkled surface, while later on it has the appearance of being sprinkled with flowers of sulphur (fig. 49). In broth delicate feathery flocculi form. Cover-glass specimens from young agar cultures show masses of tangled filaments, which appear to be more or less branched, and stain well with the ordinary anilin dyes and by Gram's method;

with the latter the filaments often appear somewhat beaded, but no trace of rosette formation or even of clubs is ever found in cultures (fig. 50). In pus, especially human, the filaments can sometimes be seen if stained by Gram's method with orange-rubin. Inoculated into the peritoneal cavity of rabbits and guinea-pigs the cultivated organism reproduces the disease, numerous actinomycotic nodules forming in the peritoneum and elsewhere. There is much doubt as to the mode of spread of, and the infection of man with, the disease. It does not seem to be particularly contagious, and diseased and healthy animals are often placed together without bad result; it can, however, be conveyed by direct inoculation, for calves inoculated intraperitoneally with portions of diseased tissues die after some weeks or months, with an abundant development of actinomycotic nodules, as shown by the experiments of Jone and Pontick. Crookshank has also infected a calf with the material from a human case. Feeding experiments give negative results. The view generally held is that the organism occurs on cereals, straw, or roots, and gains access to the system through slight scratches or wounds in the mucous membrane of the mouth, pharynx, or larynx. In man no source of infection has been traced, though one or two cases are reported where the disease has occurred after eating grains of barley. The disease is met with not only in cattle, but also in horses and swine. In the last-named animals considerable calcification may be present in the nodules, and it may be necessary to decalcify with dilute nitric or hydrochloric acid before the rosettes can be stained.

It is important to note that tuberculin may cause a reaction in actinomycosis, similar to that which occurs in tuberculosis, and as the actinomycotic lesions are very like those which are found in the latter disease, mistakes may easily be made, and can only be avoided by a microscopical

examination. It is of considerable practical importance to distinguish actinomycosis from tuberculosis, for in many cases of the former, both in man and in animals, iodide of potassium exerts a specific curative action.

'Farcin des bœufs,' a disease of cattle occurring in Guadeloupe and characterized by infection first of the skin and afterwards of the lymphatic glands and viscera, is due to a streptothrix (*S. Nocardii*¹). Gasperini² recognizes three forms of actinomyces, *Actinomyces bovis* or *sulphureus*, *Actinomyces albus*, and *Actinomyces luteo-roseus*. Homer Wright,³ however, considers that but one species of micro-organism is the ætiological agent, both in man and animals, the *A. bovis*.

From a case of brain abscess Eppinger isolated a streptothrix.

CLINICAL EXAMINATION.

1. Pour out the pus or discharge into a large capsule or Petri dish so that it forms a thin layer, look for any yellowish or other granules, pick them out with a needle, and place on a clean slide in a drop of 50 per cent. glycerin. If no granules can be found, a little of the discharge may be spread on a slide with a drop of 50 per cent. glycerin. Cover with a cover-glass and apply a little pressure. Examine with a $\frac{3}{8}$ -inch objective. If any actinomycotic tufts are present they will be seen as yellowish or pale brownish, spheroidal, ovoid, or reniform masses, and with a $\frac{1}{6}$ -inch objective will be found to have a radiating structure from the presence of the clubs.

2. Stain cover-glass specimens of the discharge, by Gram's method, with eosin. The actinomycotic tufts will generally be found to consist of little masses of tangled filaments stained violet and surrounded by a pink zone which has an indistinct radiating structure.

N.B.—In most instances the clubs in *Actinomyces hominis* do not stain by Gram's method. The reverse is the case in *Actinomyces bovis*.

¹ *Ann. de l'Inst. Pasteur*, ii., 1888, p. 293.

² *Atti Soc. Tosc. Sci. Nat.* x., 1896, p. 144.

³ *Publications of the Massachusetts General Hospital*, i., 1905 (also *Journ. Med. Research*, 1905).

3. Sections of actinomycotic tissue are best prepared by the paraffin method. If frozen, the actinomycotic nodules are very apt to fall out. Sections may be stained in any of the following ways:

(a) By Gram's method, with eosin or orange-rubin.

(b) With the Ehrlich-Biondi triple stain. Stain for from half an hour to two hours. Place in methylated spirit until the sections appear greenish, then pass through absolute alcohol and xylol. The clubs are stained yellowish-brown, and are well shown in human cases when unstained by Gram's method.

(c) By Plant's method. Stain in warm carbol-fuchsin for ten minutes, rinse well in water, stain in a saturated solution of picric acid in methylated spirit for five to ten minutes, rinse well in water, place in 50 per cent. alcohol for ten minutes, pass through absolute alcohol and xylol.

(d) Good preparations may be obtained by staining in Ehrlich's hematoxylin and counter-staining with orange-rubin. This may also show the clubs when they are unstained by Gram's method.

MADURA DISEASE OR MYCETOMA.

Madura disease, otherwise known as madura foot, mycetoma, or the 'fungus disease of India,' is a chronic local affection generally attacking the foot, occasionally the hand, sometimes extending up the leg, but rarely to the trunk. The disease occurs in certain districts in India, and full descriptions of it have been given by Vandyke Carter and by Lewis and Cunningham. A 'madura' foot appears enlarged, and numerous sinuses with raised mammillated apertures open on the surface (fig. 51). On making a section into the diseased tissues the bones are found to be more or less carious, while the soft structures are tough and hypertrophied from the occurrence of chronic inflammatory changes. Numerous small cavities are present, sometimes filled by yellowish granules resembling fish-roe, and hence termed 'roe-like particles,' at others containing black particles of irregular shape, coal-like consistence, and variable size, exceptionally as large as a marble or walnut. The presence of the white or black granules, which may be discharged from the

sinuses before mentioned, divides the disease into two classes—the so-called white and black varieties. Lewis and Cunningham have also described a third variety in which the granules are red like cayenne pepper.

Vandyke Carter¹ first called attention to the similarity between the white variety and actinomycosis in their microscopical characters. In sections stained by Gram's method more or less crescentic or reniform bodies are noticeable, divided into wedge-shaped areas, which contain masses of fine filaments stained purple. Surrounding the crescentic bodies is a zone of radially arranged elements, many of which are fan-shaped owing



FIG. 51.—A FOOT AFFECTED WITH MADURA DISEASE.
(White variety.)

to branching; they are indistinct, as they do not stain with the gentian violet, but they are very suggestive of the club-shaped structures present in actinomycosis, and they resemble the *Actinomycosis hominis* inasmuch as they do not stain by Gram's method (Plate XVIII., *b*). By staining with hæmatoxylin and orange-rubin, or with the Ehrlich-Biondi triple stain, here and there in the radial zone well-defined clubs can be demonstrated. It seems therefore that the radial zone is composed of degenerate club-shaped structures, and the disease evidently closely resembles

¹ *Bombay Med. and Phys. Soc.* ix. 1886 (new series), p. 86. Also Hewlett, *Trans. Path. Soc. Lond.* xlii., 1893.

MANUAL OF BACTERIOLOGY

mycosis, but seems to be due to a different species of streptothrix.

From a case of the white variety Boyce¹ cultivated a streptothrix which differed somewhat from the *Actinomyces*, as it grew without producing any pigment, and on agar formed white raised colonies with radial grooves, not unlike the tiny barnacles found on wooden piles in the sea. Vincent² also isolated a streptothrix, which is identical with that of Boyce, which differed from the *Actinomyces* in growing feebly in broth, in not liquefying gelatin, and in not being inoculable in the rabbit. He describes colonies on glycerin agar umbilicated colonies, first white and afterwards red. Shattock³ suggests that the red, cayenne-like grains occasionally met with in mycetoma may be the colonies of the streptothrix which have produced their color. Microscopically, this organism (*Streptothrix madura*) is identical with the *Actinomyces*. Musgrave and Clegg in a case of the white variety isolated a streptothrix (*S. freeri*) differing from *S. madura*.

The relation of the black to the white variety of *madura* has been somewhat debated. Kanthack⁴ described the

membrane, in colour pale brown with white periphery. Small drops of brown, coffee-coloured fluid appeared on the surface, and the potato became brown throughout. On agar the growth formed a meshwork of widely spreading greyish filaments; in old cultures (also in potato infusion) black hard granules, or 'sclerotia,'² were observed. In broth little balls of radiating filaments developed.

Paltauf¹ ascribed the white variety to one of the higher fungi, but since the cultivation of the streptothrix this view is not tenable. Le Dantec² met with a case of the black variety in Senegal from which he cultivated a small bacillus, but either this was a contamination or the disease was distinct from mycetoma. The writer (*loc. cit.*), in an examination of three specimens of madura disease, met with one which could not be classed with either the white or the black variety, and in which structures suggestive of a sclerotium were present.

It would seem that there are probably several conditions, both in actinomycosis and in mycetoma, having a general resemblance but differing slightly, and dependent upon different species of streptothrix.

MYCOSIS TONSILLARIS (MYCOSIS PHARYNGIS LEPTOTHRICIA).

A chronic disease attacking young adults, resistant to treatment, and characterized by the presence of small, white, tough, adherent excrescences on the mucous membrane of the pharynx. Microscopically, the patches consist of collections of epithelial cells and debris, infiltrated with leptothrix filaments and bacteria. The disease, however, seems to be a keratosis, infection with the organisms being secondary.

But occasionally a true 'mycosis' apparently occurs, readily amenable to treatment and due to a leptothrix.³

LEPTOTHRIX BUCCALIS.

Four somewhat similar thread forms occur in the mouth, viz. *Leptothrix racemosa*, *L. buccalis maxima*, *L. innominata*,

¹ *Semaine médicale*, 1894, p. 298.

² *Arch. de méd. navale*, Dec. 1894.

³ See *Glasgow Medical Journal*, No. 2, 1896, p. 81 *et seq.* (Brown Kelly).

MANUAL OF BACTERIOLOGY

Bacillus maximus buccalis. The first is very common, large threads, shows a peculiar beaded appearance on drying, which has been regarded as sporulation, and may be a true form. *L. buccalis maxima* and *L. innominata* differ from each other in that the former gives a blue granulose reaction when treated with iodine and dilute sulphuric acid, while the latter does not. All these three organisms are very similar, the filaments are either unsegmented, or the segments are of considerable length. The *B. maximus buccalis* is very like the *B. buccalis maxima*, but does not give the granulose reaction, and its segments are shorter. It is motile, flagellated, and stains by Gram's method.

Some confusion exists respecting the thread forms of the

CLADOTHRIX DICHOTOMA.

This organism is not unfrequently met with in natural waters. It forms long threads, straight, or sometimes slightly undulating, sometimes spiral and apparently branched, though the branching is automotous. It can be cultivated on the ordinary laboratory

CHAPTER XVI.

THE BLASTOMYCETES.

Saccharomycetes and *Torulæ*—The Common Yeasts and their Examination —The Pathogenic *Blastomycetes*.

The *Blastomycetes*, or yeasts, are sharply distinguished from the bacteria by their mode of reproduction. Whereas in the bacteria reproduction is by fission or simple division, in the *Blastomycetes* it is by gemmation or budding. If a cell of ordinary brewer's yeast be watched under conditions favourable to growth and reproduction, it will be found that a slight protuberance makes its appearance at one pole of the organism; this increases in size, and ultimately a daughter-cell resembling the parent is reproduced and separates off.

In some of the yeasts there is also a method of reproduction by endospore formation, and according as this occurs, or not, the *Blastomycetes* are divided into two groups:

- | | | |
|---------------|---|--|
| Blastomycetes | { | 1. <i>Saccharomycetes</i> , or true yeasts, in which spore formation occurs. |
| | { | 2. <i>Torulæ</i> , in which no spore formation has been observed. |

Although the term 'torula' has thus a definite signification, it is often loosely used to denote any yeast cell.

In addition to reproduction by gemmation, the *Blastomycetes* are also distinguished from the Bacteria by their larger size, and in those forms in which endospores occur by the

MANUAL OF BACTERIOLOGY

being multiple and not single in each cell. From the hyphomycetes, or moulds, the Blastomycetes are distinguished by being unicellular, and by the reproduction being asexual. The Blastomycetes, however, are probably more nearly allied to the Hyphomycetes than are the yeasts, for many of the moulds have a stage in which the mycelium (see next chapter) resembles an aggregation of cells, and the yeasts in old cultures form films in which the cells become much elongated, like those in the mycelium of a mould. Jörgensen and others have attempted to show that some of the yeasts are stages in the development of a fungus, but it cannot be said that this has yet been satisfactorily demonstrated.

FERMENTATION.

The yeasts are of great importance in inducing many of the changes, especially alcoholic fermentation, beer and wine-making almost exclusively due to their activity.

of plaster of Paris, the cells develop spores. First the cell becomes divided by the development of membranes, the so-called 'partition-wall formation,' into several chambers in which the spores form. In the different yeasts the number and arrangement of the spores vary; in the *S. cerevisiae* the typical number is four, arranged close together, three on one plane and one resting on these, like a pyramid of billiard balls.

The spores are of considerable importance in the identification of species of Blastomycetes, as the form of the cells alone and the growths on culture media are not sufficiently distinctive. In fact so little can these two characters be relied upon, that in order to isolate in pure cultivation it is necessary to grow from a single cell. This can be done by making a miniature plate cultivation with wort-gelatin on a large cover-glass (ruled in squares) which has been sterilized, and, after the layer of gelatin has set, mounting, gelatin downwards, on a large cell on a glass slide. The preparation is then carefully examined with a $\frac{1}{4}$ or $\frac{1}{8}$ inch objective, and the positions of single isolated cells are noted. This is not a difficult matter on account of the comparatively large size of the yeast cells, and their position is determined by the cross-lines ruled on the cover-glass. The preparations are kept in a moist chamber in a warm place, and when visible colonies have developed, those which are derived from a single cell can be inoculated into tubes or flasks of a suitable culture medium.

It is found that the various yeasts form spores in different periods of time when grown under similar conditions, and on this fact is based what is known as the analysis of yeast—a most valuable method, which we owe to Hansen. The chief 'diseases' of beers and yeast—i.e. abnormal fermentations giving rise to inferior products—are due to admixture of certain 'wild yeasts,' as they are termed, with the brewer's yeast, chiefly the *S. ellipsoideus* and *S. pastorianus*; and, in order to detect these 'disease' species, the analysis consists in determining at what time ascospores appear. The mode of procedure is as follows:

The yeast is sown in a flask of sterile wort, and incubated at 25° C. for twenty-four hours. The yeast revives, and from the deposit of young cells two cultures are made on plaster blocks. These cultures are kept, one at 25° C., the

MANUAL OF BACTERIOLOGY

and are examined twice daily. In an uncontaminated yeast ascospores should not be detected in less than 48 hours in the culture kept at 25° C., and seventy-two hours kept at 15° C. The plaster of Paris blocks are sterilized by careful flaming in the Bunsen, and are then placed in sterile capsules with lids, sufficient sterilized water being poured to thoroughly moisten the whole of the blocks; unless this is done no growth occurs. By this method of analysis as little as one two-hundredth of the whole can be detected. In addition to distinct species of yeasts there are also a number of varieties employed in brewing &c., differing but not in morphological and cultural characters, yet producing different results. These varieties may be divided into two groups—*the surface*, high or top; and the *sedimentary*, low or bottom fermentation forms. In this country beer is brewed by fermentation of malt with yeast which, during the process, rises *to the surface*, and belongs to the first group; while the *bottom* beers are obtained by yeast which, during fermentation, settles *to the bottom*, and belongs to the second group. The action of the yeast in the high fermentation process seems to be due to the attachment of a yeast bud to a surface, and the

The yeasts of the cerevisiæ group can invert cane sugar, select dextrose from levulose, and ferment maltose, but they cannot ferment lactose, nor decompose malto-dextrin.

PASTORIANUS GROUP.—These are wild yeasts. The cells are elongated or sausage-shaped, and six or eight ascospores are produced in a cell.

S. pastorianus I.—A bottom fermentation yeast producing a bitter taste in beer.

S. pastorianus II.—A feeble top fermentation form. Surface cultures on yeast-water gelatin have smooth edges, which distinguishes it from the next species :

S. pastorianus III.—A top fermentation form producing turbidity in beer. Surface cultures on yeast-water gelatin have woolly margins.

ELLIPSOIDEUS GROUP.—These are wild yeasts. The cells are usually ovoid or pear-shaped, sometimes round, rarely elongated.

Five or six ascospores are produced in a cell.

S. ellipsoideus I.—A bottom fermentation yeast occurring on ripe grapes.

S. ellipsoideus II.—A bottom fermentation yeast causing turbidity in beer.

Both the pastorianus and ellipsoideus groups resemble the cerevisiæ group in their chemical actions, but they are able in addition to decompose malto-dextrin.

S. anomalus is a yeast forming small ovoid cells. It is curious in that the spores are hemispheres with a projecting rim at the base like a bowler hat.

Another point in the identification of species of yeasts is the period of formation of films. If the yeast is grown in wort with free access of air and is undisturbed, after a varying period a film composed of a zoogleea mass of cells appears on the surface.

If yeast, or disintegrated yeast cells, be injected into animals, the blood acquires specific agglutinative properties, agglutinating the yeast cells of the species with which the inoculation has been carried out.¹

Literature on the Yeasts of Fermentation.

Jørgensen, *Micro-organisms and Fermentation*, 3rd edit. (Macmillan & Co.) (Full Bibliog.); Klöcker, *Fermentation Organisms*.

¹ See Macfadyen, *Centr. f. Bakt.* (1^{re} Abt.), xxx., 1901, p. 368.

MANUAL OF BACTERIOLOGY

EXAMINATION OF YEASTS.

Yeasts can be readily examined in the fresh state in drop preparations. The cells should be young or they be of the typical form; a two or three days old culture or grape-sugar may be used. The yeasts grow well on ordinary gelatin, agar, and potato, but wort gelatin or agar preferred. The elongated cells, common to all old cultures, may be obtained from the films which form on tubes in wide flasks or beakers after two or three weeks.

Order to stain yeasts, a dilution of the culture should be on a watch-glass of water, so that the cells may be isolated, become distorted if groups form in the preparations.

If the yeast has been grown in wort, it is best, before staining, pour off the fluid from the deposit of cells at the bottom of flask or test-tube, add some physiological salt solution, wash, then allow the vessel to stand for an hour for the sediment, and the process of washing may be repeated. Cover glass specimens may be prepared in the ordinary way and stained for five minutes in Löffler's methylene blue, in water, dried, and mounted. On the cover glass

cultivated yeast forms from fermenting fruits which, on inoculation into guinea-pigs, produced death in about a month with the formation of a tumour at the seat of inoculation and embolic growths in the spleen and liver. He also obtained a similar yeast from an ox affected with carcinoma, which on subcutaneous inoculation killed guinea-pigs in about two months, and inoculated into the peritoneum in a month, with multiple embolic growths in the lungs, spleen, and mesenteric glands. A good deal of calcification was present in the growths, from which fact Sanfelice named this yeast *Saccharomyces litogens*. Rabinowitch and also Foulerton¹ have found that some of the ordinary yeasts give rise to a tumour formation on inoculation, especially in the rabbit.

Curtis² obtained a yeast from an apparently myxomatous tumour in a young man. The organism was met with in two forms, free and encapsuled. The free form appeared in young agar cultures as round or ovoid cells measuring 3 to 6 μ in diameter, often showing budding. The encapsuled form was met with in the original tumour and in the tissues of inoculated animals, and occurred as a large sphere 16 to 20 μ in diameter, enclosing the yeast cell, the capsule being hyaline and 4 to 6 μ in thickness. On agar at 37° C. it formed whitish, opaque, creamy colonies in two to three days, becoming a thick creamy growth at the end of a week. On gelatin it formed white colonies or growth in four to five days without liquefaction, and in broth a flocculent deposit, the broth remaining clear. It was aerobic, did not grow on serum, and formed a small quantity of acetic acid and alcohol when grown in beer-wort and sugar solutions. It was not pathogenic for guinea-pigs, but inoculated into rabbits, rats, mice, and dogs it produced tumours and caused death. The tumours

¹ *Journ. Path. and Bact.* vi., 1899, p. 37.

² *Ann. de l'Inst. Pasteur*, x., 1896, p. 449 (Refs.).

to the naked eye appeared to be myxo-sarcomata, and in them the yeasts were found.

Busse also obtained a pathogenic yeast from a young woman who suffered from a tumour of the tibia, and ultimately died with diffused growths in the bones and organs. The yeast-like cells were observed in the affected parts, and were isolated by cultivation, and the cultures, inoculated into mice and rabbits, produced death with growths in the organs. As in Curtis's case, the cells in the tissues appeared to be encapsuled.

Gilchrist described a case of blastomycetic dermatitis. Small miliary abscesses were present in the rete and corium, in the pus of which the parasitic cells were observed. These were usually in pairs of unequal size, the largest measuring about $16\ \mu$, surrounded by a well-defined capsule, and containing a granular protoplasm in which a vacuole was present. Clinically, the case had been regarded as one of scrofuloderma, but no tubercle bacilli could be found.

Numerous cases of blastomycetic dermatitis have now been recognized, and several instances of general systemic blastomycetic infection have been recorded.

Granulomatous tumours occurring in epidemics among horses in Japan, France, and Italy are also caused by *Blastomycetes*.

CLINICAL EXAMINATION (PATHOGENIC YEASTS).

The cells can be well seen in the fresh state in the teased-up tissues mounted in water or glycerin.

Curtis recommends staining in carbol-thionine blue, and for sections, picro-carmin.

Busse's method for sections is as follows :

1. Hæmatoxylin solution for fifteen minutes.
2. Wash in distilled water.
3. Counter-stain in weak carbol-fuchsin (1 : 20) for thirty minutes to twenty-four hours.

4. Decolorize in 95 per cent. alcohol for fifteen seconds to one minute.

5. Absolute alcohol, xylol, mount in Canada balsam.

Gilchrist recommends treating the sections with 10 per cent. caustic potash solution and examining in 50 per cent. glycerin without staining.

Brayton recommends that small pieces of the tissues should be excised from the growing margin, treated with ether for two to five minutes, macerated in 20-30 per cent. caustic potash solution for five to ten minutes, and then examined without staining. Cultures may be readily obtained, with a little care, preferably on beerwort gelatin.

Literature on the Pathogenic Blastomycetes.

Busse, *Die Hefen als Krankheitserreger*, Hirschwald, 1897.

Gilchrist, *Johns Hopkins Hosp. Bull.* i., 1896, p. 269.

Gilchrist, *Journ. of Exp. Med.* ii., 1898, p. 56.

Roncagli, *Journ. Path. and Bact.* v., 1898, p. 1. (Bibliog.).

Brayton, *Journ. Amer. Med. Assoc.* xxxviii, 1902, p. 313.

LeCount and Myers, *Journ. of Infectious Diseases*, iv., 1907, p. 187.

CHAPTER XVII.

THE HYPHOMYCETES—RINGWORM AND THRUSH.

The Hyphomycetes are an important group of the true fungi, and include those forms which are commonly known as moulds. They are multicellular individuals, composed of filaments which may be simple or branched, jointed or unjointed. These filaments are termed hyphæ, and are formed by the end-to-end union of elongated cells. When the hyphæ project upwards into the air they are known as aërial hyphæ, and when downwards into the fluid or medium on which the organism is growing, as submerged hyphæ, and the compact tufts or masses resulting from numbers of interlacing hyphæ are termed mycelia. A mycelium may form a hard lignified mass or pseudo-parenchyma, which is known as a sclerotium, such as is met with in ergot and in the black variety of mycetoma. In addition to being multicellular, the higher development of the Hyphomycetes is seen in the specialization of certain parts for the function of reproduction, reproductive organs being produced. Although all the species multiply asexually, in most, if not in all, a sexual method occurs also. *Mucor mucedo*, *Penicillium glaucum*, and *Aspergillus niger* may be taken as types and more fully described.

MUCOR MUCEDO.

Mucor mucedo, the common white mould which appears like tufts of cotton-wool on various substances, may be obtained by

exposing some moistened bread or horsedung to the air for a short time, and then keeping it moist under a bell-jar. It consists of a mycelium composed of hyphæ, and its fluffy appearance is caused by aerial hyphæ. The aerial hyphæ are at first of even diameter throughout, but later on their free ends become swollen and ultimately form spherical bodies, which become filled with spores and are known as sporangia. In the early stage the whole organism forms but a single cell, the protoplasm of which is granular, and contains vacuoles and numerous small nuclei. As it grows, and the sporangia form, these become separated by a septum from the hyphæ, and when it becomes older still the mycelial hyphæ may be divided into elongated cells. The development of a sporangium takes place as follows: The distal end of an aerial hypha swells, and immediately below the swollen part a division occurs in the protoplasm and a cellulose septum is formed, so that the swollen part is separated off from the rest of the hypha, forming the rudimentary sporangium. The sporangium continues to grow, and its protoplasm undergoes multiple fission into numerous ovoid masses, the spores, each of which becomes surrounded with a cellulose capsule. The septum separating the sporangium from the hypha projects upwards into the interior of the sporangium as a club-shaped knob known as the columella. When the sporangium is ripe the slightest touch causes its wall to rupture, so liberating the spores. When placed under favourable conditions the spore germinates, it becomes granular, and bulged out in one or more places, and these buds increase in length and ultimately form hyphæ.

Occasionally a process of conjugation occurs. Two adjacent hyphæ send out lateral branches which come in contact with one another, and a septum forms in each, separating a small portion of protoplasm from the rest of the hypha. The apposed walls of the two cells become absorbed and the contents mingle. The mass of protoplasm so formed becomes surrounded with a thick cell-wall, giving rise to an inactive spore-like body known as a zygospore. The zygospore under favourable conditions sprouts like an ordinary spore, forming a mycelium which develops sporangia bearing aerial hyphæ.

Certain mucors form appreciable amounts of alcohol.

MANUAL OF BACTERIOLOGY

PENICILLIUM GLAUCUM.

forms the bluish-green mouldy patches familiar to every one. It is by far the commonest of all species, and may be obtained from bread or jam or by exposing a gelatin plate to the air for some time. If the mouldy patch be rubbed a fine greenish dust comes away. This dust consists of myriads of spores; if it be transferred with a moistened needle to a gelatin plate, or better still, to a hanging-drop preparation, the growth of the organism can be studied. After two or three days little white tufts will be observed, which microscopically are found to consist of tufts of delicate interlacing filaments, the hyphæ; becoming much interwoven, ultimately form a tough mass. The patches of growth are circular, and the hyphæ tend to radiate from the centre. As the patch increases it changes in colour, becoming bluish-green, though the centre for some time still remains white. From the upper surface of the mycelium delicate aerial hyphæ grow upwards, and from the under surface short submerged ones project downwards. The hyphæ are composed of elongated cells arranged end to end, the cell-walls of which consist of cellulose enclosing a more

walls a sclerotium form arises ; it is a hard solid body, yellowish in colour and resembles a grain of sand, the carpogonium being at the centre. If placed in favourable conditions the sclerotia germinate after some time. Two forms of hyphæ are produced, one thick, the other thin ; the latter become much twisted. The thick hyphæ become branched, and ultimately a number of pear-shaped bodies are produced. The contents of these bodies then become broken up and form spores ; the bodies are known as asci and the spores as ascospores. From the ascospores the ordinary mycelial form again develops.¹

ASPERGILLUS NIGER.

Aspergillus (several varieties) is occasionally met with ; it can be recognized by the rounded sporangia with radial markings which are supported on aerial hyphæ given off from the mycelium. A process of sexual reproduction occurs very like the one observed in penicillium. *Aspergillus niger* grows well on the ordinary laboratory media, producing on potato a powdery, sooty growth after a time.

With the exception of the ringworm and allied fungi, which produce parasitic skin affections, the Hyphomycetes are not of very great pathological importance. In the ear and nose, mucors and aspergilli may be met with, but in these situations they are epiphytes rather than parasites, and the same species occur in bronchiectases and pulmonary vomicæ. Occasionally, however, a pneumono-mycosis² has been met with, the mycelium of the fungus ramifying in the lung tissue and setting up irritative and other changes. 'Pneumono-mycosis' or 'pulmonary aspergillosis' is especially a trade disease among bird-rearers. Grain is taken into the mouth and the bird fed with it, and in the course of this operation the mould spores are inhaled. The course

¹ See Brefeld, *Quart. Journ. Microscop. Soc.* xv. p. 342.

² Arkle and Hinds, *Trans. Path. Soc. Lond.* vol. 47, 1896, p. 8 (Bibliog.) ; Boyce, *Journ. Path. and Bact.* Oct. 1892 (Bibliog.).

of the disease is much like chronic bronchitis or pulmonary tuberculosis. The species met with in this condition seems generally to have been the *Aspergillus fumigatus*.

The black variety of madura disease, as already stated (p. 434), is due to a fungus form.

CULTIVATION AND EXAMINATION.

The Hyphomycetes can be cultivated on the ordinary laboratory media, but wort-agar, or wort-gelatin, potato, bread, or maltose agar is to be preferred.

They can be examined by removing a portion of the growth, teasing up gently with needles in a little 50 per cent. alcohol containing a trace of ammonia, removing the surplus fluid with blotting-paper, and mounting in Farrant's solution or in glycerin jelly. If desired, they may be stained by the irrigation method with fuchsin.

In the tissues they may be stained with hæmatoxylin or methylene blue, or by Gram's or by Weigert's method.

RINGWORM.¹

The ringworm fungi must probably be included in the group of the Hyphomycetes. Human ringworm, formerly regarded as a single disease, has been proved to comprise at least two affections through the researches of Sabouraud. These two forms are distinguished from each other clinically and by differences in the parasitic organisms.

The first variety is an affection of early childhood, forming 80 to 90 per cent. of the ringworms met with in London; it never attacks the scalp of adults, never affects the beard or nails, is very intractable, and frequently epidemic. The parasite is characterized by small spores

¹ On Ringworm, see Colcott Fox and Blaxall, *Brit. Journ. of Dermat.* 1896 (Bibliog.), and *Trans. Path. Soc.* vol. 48, 1897, p. 301; also Malcolm Morris, *Trans. Internat. Congress of Dermatology*, 1896. Also *Brit. Med. Journ.* 1897, ii.

measuring 3 to 4 μ in diameter, and round or ovoid in shape. Affected hairs are generally broken off, forming relatively long stumps, greyish in colour, and possessing a whitish sheath. When suitably prepared in potash, this sheath is seen to be composed of the spores agglomerated together without apparent order, and the hairs themselves are filled with delicate parallel mycelial threads (fig. 52). The parasite is named the *Microsporon Audouini*.

The second variety comprises the ringworms with large spores, and is divided into two groups by Sabouraud. The

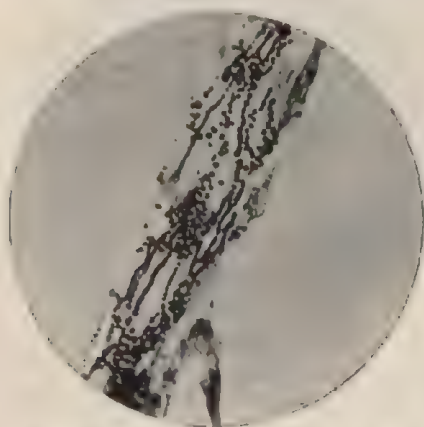


FIG. 52.—RINGWORM IN A HAIR. $\times 350$.

first of these groups is exclusively of human origin, and has a marked tendency to affect the interior of the hair only, and hence the parasite has been termed the *Trichophyton megalosporon endothrix*. The other group is of animal origin, and the spores are met with chiefly on the outside of the hair, and the fungus is hence termed the *Trichophyton megalosporon ectothrix*.

The *endothrix* form occurs later in childhood, is not so persistent as the *microsporon*, and does not attack the nails

or beard. Microscopically, the fungus is seen to consist of beaded threads, which are rounded or ovoid spores joined end to end. The *ectothrix* form rarely attacks the scalp, but is responsible for all the tinea sycosis and ringworm of the nails and half the cases of tinea circinata. Suppuration is common in this form. Microscopically, appearances differ; generally the spores are arranged in chains, but the sporulation is less regular than in the *endothrix*. The spores in the *endothrix* and *ectothrix* varieties measure 4 to 12 μ in diameter.

The ringworm fungi can be readily cultivated on all the ordinary media—beer-wort agar and beer-wort gelatin being especially favourable. They form whitish fluffy growths with rapid liquefaction of gelatin. In order to obtain cultivations the diseased hairs or stumps are removed by forceps and placed on a sterile glass slide. The aërial portion of the hair is then cut away by means of a sterile scalpel, and the diseased portion divided into small fragments. These can be picked up with a moistened platinum needle and transferred to the culture media, preferably beer-wort agar. In some cases a pure culture is thus obtained, but in others further treatment is necessary. When the *Trichophyton* or *Microsporon* has thrown up its aërial hyphæ the plug of wool is removed from the tube and the mouth well flamed; the tube is then held inverted over a Petri dish containing solidified maltose agar. A sharp tap or two is given to the tube, sufficient to cause the spores to drop, and the dish re-covered. A growth of the organism from single isolated spores thus ensues, and pure cultures can be obtained (Blaxall).

The various forms of the ringworm fungi can be differentiated by cultures, but it is necessary when comparing them to employ media of identical composition, because slight differences in the latter are liable to induce marked changes in the characters of the cultures. A favourite

medium, used by Sabouraud and by Blaxall, is maltose agar :

Peptone	0.5 gram
Maltose	3.8 grams
Agar-agar	1.3 grams
Water	100 c.c.

Blaxall found that different maltoses materially influenced the characters of the cultures.

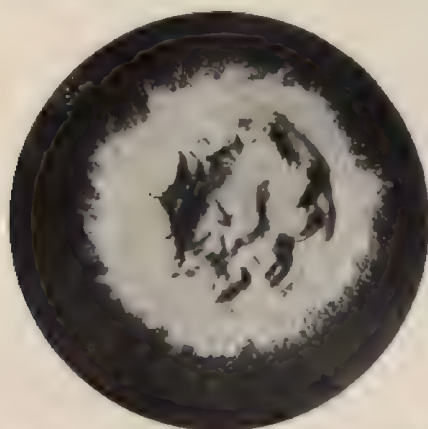


FIG. 53.—CULTURE OF THE RINGWORM ORGANISM.
ENDOTHRIX FORM.

Characters of the Cultures.—Cultures are incubated at 30° C. The colonies of the *Microsporon* do not show any growth until about the seventh day ; little white downy tufts then appear. The fully developed growth on maltose agar forms a large white downy patch with a small central boss ; on potato, white downy patches with brown discoloration.

The *endothrix* variety commences to grow in six or seven days, and on maltose agar in about a month forms a rounded patch with a central crateriform depression, the whole being dusted with fine white powder (fig. 53) ; on

MANUAL OF BACTERIOLOGY

powdery stars develop tinged with yellow and usually discoloration of the medium.

Cultures of the *ectothrix* form are variable. They appear on the third or fourth day; some develop whitish, or wrinkled growths; others, from the dog, form open, wrinkled, powdery growths; others, of bird origin, form purplish growths.

Microscopically, all the fungi show masses of mycelial threads with spores. They stain with the ordinary aniline dyes, also by Gram's method, and can be mounted in glycerine jelly in the manner described at p. 450.

Wiedemann¹ found that the ringworm organism produces a peptonizing enzyme, and seems to increase the amount of keratin when grown on it; no inverting ferment could be isolated.

CLINICAL EXAMINATION.

Scalp diseases should be treated first with ether and then with

FAVUS.—Favus is due to a fungus discovered by Schoenlein in 1839, the *Achorion Schoenleinii*. It is seen as a mycelial growth with spores in the patches. The organism grows well on maltose agar, forming fluffy, woolly moss-like colonies with radiating outgrowths, first grey, and then yellowish. It occurs on mice and other animals.

PITYRIASIS VERSICOLOR.—In the epidermal scales of this skin affection a fungoid organism (*Microsporon furfur*) is present. It occurs as short and thick curved hyphæ between which are masses of large coarse spores. It has not been cultivated.

PINTA.—A skin disease met with in South America. In the scales short mycelial filaments with large (8-12 μ) spores are seen. Various organisms have been cultivated belonging to the genera *Penicillium* and *Aspergillus*.

PIEDRA.—A disease of the hair met with in South America. The nodosities on the hairs are composed of masses of very large refractile spores.

THRUSH.¹

Thrush is due to an organism (*Oidium albicans*) which is usually classed among the Hyphomycetes. It forms the whitish patches so frequently seen on the mucous membrane of the mouth and pharynx in children, and in those suffering from wasting diseases, but a general infection has occasionally been produced by it. If one of these patches is removed and teased up, it will be found to consist of masses of tangled mycelial threads with yeast-like budding. The organism can be readily cultivated on all the ordinary laboratory media, and will also grow on slightly acid media such as wort-gelatin. It produces whitish, membranous, adherent growths in which it appears morphologically under two forms, as masses of tangled filaments or hyphæ and as yeast-like cells. On acid media the latter exclusively occur, on alkaline the former predominate. It liquefies gelatin, stains by Gram's method, produces an alkaline reaction by the formation of ammonium carbonate, and does

¹ See Teissier, *Arch. Méd. Expér.* ix., 1897, p. 253.

CLINICAL

The patches may be teased
described for the Hyphomycete
may be stained with carbol-fuc

CHAPTER XVIII.

THE PROTOZOA.¹

The General Structure of the Protozoa—Pathogenic Amœbæ—Trypanosomata—Leishman-Donovan Body—Spirochaetæ—Coccidia—Malaria.

The Protozoa are an important group of unicellular organisms, regarded as animal in nature, and sharply and definitely distinguished from the rest of the animal kingdom, to which the names of metazoa and enterozoa are applied. The latter consists of many cells, differentiated to perform different functions, and arranged in two layers—endoderm and ectoderm—around a central cavity, the enteron.

‘It is true that some protozoa consist of aggregates of cells, and should therefore be entitled to be called multicellular; yet an examination of the details of structure of these cell-aggregates and of their life-history establishes the fact that the cohesion of the cells in these instances is not an essential feature of the life of such multicellular protozoa, but a secondary and non-essential arrangement. Like the budded “persons” forming, when coherent to each other, undifferentiated “colonies” among the polyps and corals, the coherent cells of a compound protozoon can be separated from one another and live independently; their cohesion has no economic significance. Each cell is precisely the counterpart of its neighbour; there is no common life, no distribution of function among special groups of the associated cells, and no corresponding

¹ See Minchin in Ray Lankester's *Text-book of Zoology*, and Clifford Allbutt's *System of Medicine*, ed. 2, vol. ii. Pt. ii.; Hartog in *Cambr Natural History*, vol. i.

MANUAL OF BACTERIOLOGY

of structure. As a contrast to this, we find the simplest enterozoa that the cells are functionally and structurally distinguishable into two groups—those which line the enteron or digestive cavity, and those which form the outer body wall. The cells of these two layers are interchangeable, but are fundamentally different in function and structure' (Ray Lankester). It is true that in some instances there may be a difficulty in deciding whether an organism is vegetable or animal, and Haeckel is inclined to include all unicellular organisms in a distinct kingdom, the Protista.

The cytoplasm of a protozoon is commonly differentiated into an outer, clearer, denser layer or ectosarc, and an inner, granular, more fluid portion, the endosarc. The organism is sometimes naked, or may be covered with a cuticle, usually protein in nature. The cytoplasm contains a prominent nucleus, sometimes a secondary nucleus, and occasionally subsidiary chromatin particles or *chromidia*.

A contractile vacuule, which is an excretory organ,

According to Bütschli, the protozoa may be divided into the following classes:

1. SARKODINA.—Protozoa in which the cell protoplasm is naked, and locomotion and ingestion of food are performed by means of *temporary* protoplasmic processes or pseudopodia. It includes the Amœbæ, Foraminifera, and Radiolaria.

2. MASTIGOPHORA.—Protozoa in which the cell protoplasm is usually enclosed within a cuticle, and which have one or a few locomotive organs in the form of flagella. It includes the trypanosomes, *Noctiluca*, *Trichomonas*, and perhaps the spirochaetes.

3. SPOROZOA.—Protozoa which are exclusively parasitic, possessing neither flagella nor cilia, and usually not amœboid. Reproduction by sporulation, binary fission being almost unknown in this group. It includes the malaria and other important parasites.

4. INFUSORIA.—Protozoa possessing locomotive organs in the form of cilia, and in which the nuclear apparatus is differentiated into a vegetative *macronucleus* and a generative *micronucleus*. Many common animalcules, such as *Paramecium* and *Vorticella*, belong to this group.

CLASS I.—SARKODINA.

The Sarkodina includes a number of forms of very varied morphology and habits, such as the Amœbæ, Heliozoa, Radiolaria, and Foraminifera; the three latter groups being characterized by the presence of a siliceous or calcareous skeleton or shell.

PATHOGENIC AMŒBÆ.¹

Three species of *Amœba* seem to be parasitic in man, and the generic name of *Entamoeba* has been given to

¹ Councilman and Lafleur, *Johns Hopkins Hosp. Reps.* ii., 1891; Schaudinn, *A. K. Gesundheitsamte*, xix. p. 547; Strong, Musgrave, Clef Thomas and Woolley, Bureau of Gov. Laboratories, Manila, *Bulls.* 18 and

them. One, the *A. buccalis*, occurs in the mouth in dental caries, the other two inhabit the intestine. One of the latter, the *Entamæba coli* (*Amæba coli*, Lösch), occurs in the upper part of the large intestine and appears to be harmless; the other, the *Entamæba histolytica*, is regarded as the cause of one form, the amœbic, of tropical dysentery.

The *Amæba histolytica* is met with in the fæces in certain cases of dysentery, and in the pus of the so-called tropical abscess of the liver. It occurs as a large protoplasmic mass, measuring 25 to 35 μ in diameter, possessed of slow amœboid movement, and having a clearer outer zone or ectosarc and a granular endosarc. The pseudopodia are always blunt, never pointed (fig. 55). In the endosarc highly refractile granules occur, and it often contains blood corpuscles and a vacuole (fig. 54, *b*). A nucleus can also be demonstrated, but being poor in chromatin, stains with difficulty (fig. 54, *a*). According to Schaudinn, the *A. coli* differs from the *A. histolytica* in that the ectoplasm is not distinctly seen except during the formation of a pseudopodium and the nucleus stains deeply. The development of the two forms is also different. *A. coli* multiplies by simple binary fission, and also by multiple fission into eight small amœbæ. Encystment may also occur, with repeated binary division of nucleus and protoplasm, part of the nucleus being cast out, and ultimately the cyst contains eight nuclei around which the protoplasm collects, so that, if swallowed, eight small amœbæ are set free.

The *A. histolytica* multiplies by binary fission, and also by irregular gemmation, so that an indefinite number of small amœbæ is formed. Instead of encystment occurring, as in the other species, resistant spores are formed. The nucleus gives off chromidia, some of which, together with portions of the ectoplasm, are extruded and become spores surrounded by tough capsules. Apparently infection

of a fresh host occurs only with material containing these spores.

The presence of the *Amœba* in the pus, and especially

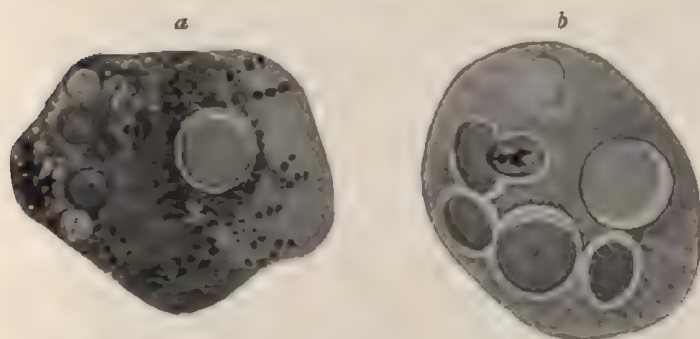


FIG. 54.—*AMŒBA HISTOLYTICA*. (After Councilman and Lafleur.)

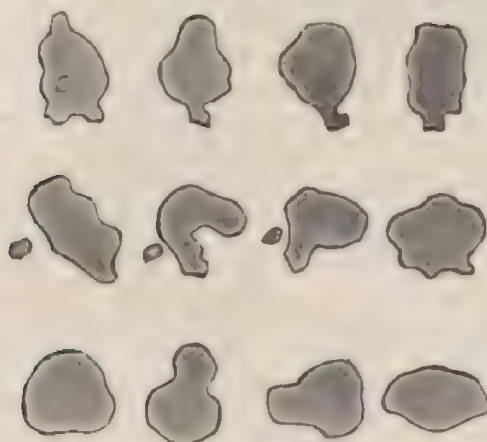


FIG. 55.—CHANGES IN FORM OF AN *AMŒBA HISTOLYTICA* OBSERVED ON A WARM STAGE, AND DRAWN AT INTERVALS OF ONE MINUTE. (Semi-diagrammatic by the writer.)

in the wall, of tropical abscesses is of considerable diagnostic significance, and the parasite is considered to be

etiological importance in cases of true tropical dysentery (see 'Dysentery'). The amœbæ are not usually observed in the abscess pus at the time of operation, but make their appearance in the discharge about the third day, i.e. when the wall of the abscess cavity is breaking down. In the true tropical abscess the ordinary pyogenic organisms are absent, unless a secondary infection has occurred, which is the exception. The abscess is usually single, and Rogers suggests that the amœbæ reach the liver by adhesions between it and the bowel. The amœbæ may be cultivated on ordinary or on water agar provided some bacterium is present at the same time, e.g. *B. coli*, cholera, or others. Material rich in amœbæ may be smeared over agar plates, which are grown at 25°-30° C. for twenty-four to forty-eight hours, and then examined with a low power. In any spot where a single amœba, or a small group, is observed, with a little dexterity the organism may be lifted up with a fine needle and transferred to a fresh plate, and by a repetition of the process, pure cultures may be obtained. The cultivated amœbæ are pathogenic for monkeys, and induce abscess on inoculation into the liver. Musgrave and Clegg (*loc. cit.*) are of opinion that all amœbæ are, or may become, pathogenic. The amœbæ are sometimes present in large numbers in the stools, being most numerous in the acute stage.

CLINICAL DIAGNOSIS.

1. A drop of the dysenteric discharge (the mucous portions should be chosen from the stools), pus, or, better, a scraping from the wall of the abscess, diluted, if necessary, with a little warm (87° C.) physiological salt solution, is placed on a slide, covered with a cover-glass, and examined microscopically with a $\frac{1}{2}$ or $\frac{1}{6}$ inch objective. The amœbæ will be readily recognized, and may be examined more critically with a $\frac{1}{2}$ -inch oil-immersion. To be certain that the bodies are amœbæ, the amœboid movements must be observed by keeping the preparation on a warm stage.

The stools should be fresh, unmixed with urine, collected in a warmed bed-pan, and kept at blood heat until examined, which should be done as soon as possible.

2. The *living* amoebae in the stools may be stained by the irrigation method with a weak ($\frac{1}{2}$ -1 per cent.) aqueous solution of neutral red. Preparations may also be stained by irrigation with methylene blue and Beale's carmine; the latter stains the nucleus, the former does not. The preparation may be rendered permanent by washing away the excess of stain, and running in some 50 per cent. glycerin by irrigation.

3. Zorn recommends the following method for preparing stained and permanent preparations: A few cubic centimeters of faeces are mixed with three or four volumes of a solution consisting of fifteen parts of 1 per cent. solution of chromic acid and three parts of 1 per cent. solution of osmic acid. The mixture is shaken thoroughly, and after ten minutes is centrifuged. The sediment is then mixed with five volumes of a 25 per cent. solution of Beale's carmine, allowed to stand for half an hour and again centrifuged. The sediment is then washed in a weak, rose-coloured solution of the same carmine, and mounted in glycerin, or first dehydrated and then mounted in balsam.

Twort¹ recommends the following method for staining sections:

To prepare the stain make up half-saturated watery solutions of neutral red and of Grüber's light green, using distilled water. Place the neutral red solution in a large open vessel, and add to it sufficient light green solution to combine with the neutral red; the compound will form a precipitate. It is better not to have an excess of either stain, as the precipitate is difficult to wash. When the neutralization point is reached, the water will contain a small quantity of both dyes in solution, giving it a dark appearance. The presence of both stains in solution can be easily tested by spotting a drop of the filtrate on to blotting-paper; this will spread out, leaving a light red central zone and a faint green outer zone.

To collect the precipitate, it is better not to filter, for the stain soon blocks the pores of the filter-paper. If the solutions are

¹ Communicated to the writer by Dr. Twort.

warmed to about 80°-40° C. before mixing, the precipitate will form large sticky masses, some of which, containing air-bubbles, float to the surface and can be removed with a spatula. The rest settle and stick to the bottom and sides of the vessel, and can be collected after pouring off the fluid. The precipitate so collected is rinsed in distilled water and dried at 87° C. In this state it forms dark greenish masses, insoluble in water, somewhat soluble in ethyl alcohol, but soluble to a greater extent in methyl alcohol, and especially so if 5 per cent. of glycerin is added.

To make up the stain for use, it is best to pound up about 0.25 gram of the stain with some clean sharp sand; this prevents the stain going into a sticky mass when the alcohol is added. To the powder so obtained is now added some purest methyl alcohol (Merck's), acetone-free, containing 5 per cent. by volume of glycerin. Pound up well to obtain a saturated solution; then pour off and add a further quantity of alcohol-glycerin solution, and repeat the pounding; about 100 c.c. stain can be made from the quantity given.

The alcohol-glycerin mixture dissolves about 0.1 per cent. of the stain, but it is always better to work with an excess of the powder when grinding up, or it is *very difficult* to obtain a *saturated solution*.

The solution, when filtered, should be kept in a good stoppered bottle (and if a completely saturated solution has been obtained add 10 per cent. more alcohol-glycerin mixture).

Tissues to be examined should be fixed in Müller's fluid containing 10 per cent. of formalin, but on no account should 10 per cent. formalin be used. Imbed tissues in paraffin, fix sections on slides, remove paraffin by xylol. Remove xylol with absolute alcohol, and place in distilled water.

Now stain for about five minutes with the stain made up by mixing one part of distilled water with two parts of the glycerin-alcohol stain solution. Sometimes in staining such organisms as glands ten minutes may be necessary, especially if insufficient stain is in solution and the room temperature is low. Rinse in distilled water.

Fix for half to one minute in Unna's glycerin-ether mixture—2 per cent. in distilled water. Rinse in distilled water.

Differentiate and dehydrate in absolute alcohol. Should there be much precipitate, this can easily be removed by a few drops of methyl alcohol, or better by a mixture of equal parts of absolute alcohol and xylol; this will remove any precipitate without over-decolorizing the tissues and micro-organisms, which is very often the case if methyl alcohol is used. Remove absolute alcohol with xylol, and mount in Canada balsam in the usual way.

Provided the necessary tissues are present, stained sections will show five distinct colours :

Purple red, presented chiefly by chromatin of nuclei. A very bright red, somewhat tinged orange, shown by mucoid and colloid degenerations. Orange red, shown by fetal cartilage and other tissues. Blue-green colour, taken up by fibrous tissue &c. Light grass-green colour, shown by red blood-cells, &c. Micro-organisms will stain bright red and will stand out in marked contrast to the green connective tissue containing them.

Animal parasites, e.g. amœbæ, also stain well. The stain has the advantage of leaving all the tissues sharply differentiated. The red does not diffuse into the tissues stained by the green, nor the green diffuse into the tissues stained by the red.

Allusion may here be made to the Mycetozoa (*Myxomycetes*). These are masses of protoplasm resembling huge amœbæ, which are found on decaying vegetable matter. By some they are regarded as vegetable, by others as animal, in nature, and belonging to the *Amœbæ* of the *Sarkodina*. Some important plant diseases, such as the 'finger and toe' of cabbage roots, are due to their activity. The finger-and-toe disease is due to an amœboid parasite (*Plasmodiophora brassicae*, by some included among the *Amœbæ*), the cycle of which begins with spores from which small flagellule are set free. Similar organisms have been supposed to be present in cancer.

CLASS II.—MASTIGOPHORA.

The Mastigophora are protozoa in which one or more permanent organs serving for locomotion or food-capture are present in the form of flagella. As a rule the body is by either a cuticle or a differentiation of the protoplasm.

firmer external portion or *periplast*. One, two, or more flagella may be present, and when multiple are arranged in various ways. Food-vacuoles may occur in the protoplasm, also contractile vacuoles, but not in the parasitic forms. Various other granules, including *chromatophores*, which generally contain chlorophyl, may be present. The nuclear apparatus is usually double, consisting of a large principal or macro-nucleus, and a small or micro-nucleus or blepharoplast; the latter is not, as in the Infusoria, composed of generative chromatin, and is in relation with the locomotor apparatus. An undulating membrane, a thin protoplasmic membrane attached to one aspect of the body like a dorsal fin, may be present. The chief parasitic genera are:

Trypanosoma and *Trypanoplasma*, both of which have an undulating membrane, but the former has one flagellum, the latter two flagella, one at each end of the body, but both starting from the blepharoplast.

Herpetomonas, like *Trypanosoma*, has a single flagellum, but no undulating membrane.

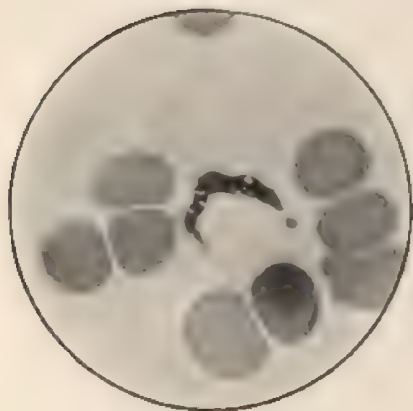
Crithidia has a pear-shaped body with single flagellum.

Trichomonas, also somewhat pear-shaped, with three short flagella and an undulating membrane.

TRYPANOSOMA.

A number of important parasites belong to this group. All of them have great morphological similarity, which renders them practically indistinguishable by structural characters. Trypanosomes can usually be differentiated into three forms—indifferent, male, and female—which in some cases may all occur together, but only become fully differentiated in an invertebrate host. The males are slender, active, only slightly granular, and with an elongated nucleus; the females are bulky, sluggish, granular, and have a rounded nucleus; the indifferent forms are intermediate. The males usually soon die off unless they conjugate; the indifferents are more hardy, the females most so. The sexual forms conjugate in an invertebrate host,

PLATE XX.



a. TETPANOROMA BRUCEI.
SMEAR OF BLOOD. $\times 1500$.



b. SPIROCHAETA RECURRENTIS (OBERMEIERI)
SMEAR OF BLOOD. $\times 1500$.



but if the males have died off, both male and female forms may be reproduced from the females by a process of parthenogenesis.

The *Tr. Brucei* is the causative parasite of nagana or tsetse-fly disease of horses in Africa. It is a somewhat spindle-shaped, wormlike organism, the posterior extremity of which is blunt, while the anterior one tapers and is prolonged into a long wavy flagellum. Along one surface of the long axis of the body a delicate wavy membrane is present, which commences at the blepharoplast and is continued into the flagellum (Plate XX., *a*). The organism is abundant in the blood of the peripheral circulation and of the organs. In the fresh blood the organism is actively motile, moving by rapid lashing movements of the flagellum (which is *anterior*), by wavy movements of the membrane, and by contractions and relaxations of the body; it can also move with the blunt end forwards. Near the middle of the parasite is a large body, the macronucleus, and between it and the blunt extremity is another smaller body, the micronucleus or blepharoplast. Near this is usually a vacuole, and the protoplasm is more or less granular. The size of the organism varies in different animals and at different stages of the disease; in an infected rat it measures 25–35 μ in length and about 2 μ in greatest diameter. Reproduction takes place by longitudinal division, possibly also by transverse division, by conjugation, and by the formation of amœboid and plasmodial masses. Of the two last named, the amœboid forms are seen in the cerebral capillaries and in the spleen, the plasmodia only in the spleen. Both give rise to the typical organism by division, which may be very rapid. The *Tr. Brucei* can be cultivated, though with difficulty, on rabbit-blood agar—melted sterile agar cooled to 45° C. + sterile defibrinated rabbit's blood warmed to 45° C., mixed, and allowed to solidify in the sloping position (Novy and McNeal).

Nagana is met with in large tracts of country in Zululand and West Africa. It especially attacks the equines—horse, mule, and ass—in which it is very fatal. The animals become anæmic and emaciated, there is a discharge from the eyes and nose, staring coat, swelling of the legs and neck, and fever. The animal dies two to six weeks after infection. Oxen are also attacked, but a small proportion recover. The dog, cat, rabbit, guinea-pig, mouse, and rat may be infected by inoculation with the fresh blood of a diseased animal. The disease is conveyed through the bites of a tsetse-fly (*Glossina morsitans*). The trypanosome is believed to live in the big game, from whence it is transmitted to horses entering the infected localities. The blood loses its infective properties usually within twenty-four hours of being withdrawn.

Surra attacks horses in Burma, Mauritius, and the Philippines, and is pathogenic to the same animals as nagana, and in the blood a parasite (*Tr. Evansi*) similar to that in nagana, but more active, was observed by Evans. Koch, Nocard, and Rogers regard these two diseases as identical, and surra is believed to be spread by certain biting flies, *Stomoxys* or *Tabanus*.

In human trypanosomiasis and sleeping sickness of West and Central Africa, a trypanosome, *Tr. Gambiense* (fig. 56),



FIG 56.—*TRYPANOSOMA GAMBIENSE*.
× 3000.

seems to be the causative agent. It is usually present, though scanty, in the blood, but can be found in large numbers in the fluid aspirated from the enlarged cervical glands.

In the later stages, when cerebral symptoms ensue, it is found in the cerebro-spinal fluid, but not in numbers, centrifugalization being necessary in order to demonstrate the parasites. The *Tr. Gambiense* is pathogenic to monkeys, and to a less extent to

white rats and guinea-pigs. It appears to be conveyed by a tsetse-fly (*G. palpalis*), and perhaps by other biting flies.

The tsetse flies (*Glossina*) belong to the house-fly order (Muscidae) and have a general resemblance to a house-fly, but when at rest the wings fold completely over each other. The proboscis is long and straight and the wing venation is characteristic, especially the fourth longitudinal vein, which makes two bends. Instead of laying eggs, the female extrudes a single full-grown larva. They are confined to Africa and Arabia; some nine species are known, and they occur in the vicinity of water on the edge of forest land ('fly-belts'). It is doubtful if any developmental cycle of the trypanosome takes place in the fly; infection is probably mainly one of direct inoculation (see p. 499).

Tr. equinum attacks horses in South America, causing weakness and paresis of the hindquarters ('mal de caderas'). Cattle are immune, most other animals susceptible.

Tr. Theileri, the largest trypanosome known (50-60 μ in length), is found in cattle in South Africa, and is not pathogenic to any other animal.

Tr. dimorphum occurs in two forms, large and small, in horses in Africa. Is pathogenic to most animals.

Dourine, a venereal disease of the horse met with in North Africa, Spain, and Hungary, is due to the *Tr. equiperdum*, which is conveyed by direct contact, and is mainly confined to the lesions, being scanty in the blood. It is pathogenic to the ordinary laboratory animals.

In rats a non-pathogenic trypanosome was found by Lewis (*Tr. Lewisi* or *sanguinis*). It is especially met with in sewer-rats, but also occurs in field-rats (Crookshank). It is somewhat shorter and thinner than the *Tr. Brucei*, and there are other small differences between the two forms. With the exception of rats and mice, and to a less extent guinea-pigs, other animals cannot be infected with the *Tr. Lewisi*. It may be kept alive for long periods in the blood placed in a refrigerator, whereas the *Tr. Brucei* soon dies under the same conditions. The two forms do not protect against each other. The *Tr. Lewisi* is readily cultivated on rabbit-blood agar and is probably transmitted by a louse.

A number of other trypanosomes have been found in the lower animals, birds, fish, reptiles, and amphibians. A large and characteristic one is generally present in the blood of the eel.

The trypanosomes are usually agglutinated when mixed with the serum from an infected animal.

EXAMINATION.

The trypanosomes, if numerous, are readily observed in the fresh blood. A very shallow cell may be formed on a slide by ringing with melted paraffin. For stained preparations the Leishman stain (see Malaria) may be employed.

Plimmer¹ recommends the following method for staining smears of trypanosomes &c. The specimen is treated while wet and before drying :

1. Expose a cover-glass to the vapour of

Osmic acid, 1 per cent.	1 c.c.
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Glacial acetic acid	8-5 drops
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for two minutes.

2. Place a drop of fresh blood in one corner of the cover-glass, and expose again to the vapour for thirty seconds.

3. Spread the film carefully, and expose again for fifteen to thirty seconds to the vapour until the surface appears no longer moist. (The film will not be really dry, and can be easily smeared off the glass with the finger : a really dry film will be much lighter in colour, and cannot be rubbed off with the finger.)

4. Place the cover-glass in absolute alcohol for ten minutes.

5. Place the cover-glass in a faintly rose-coloured solution of permanganate of potash for one minute. (Two or three drops of a 1 per cent. solution to 50 c.c. of water.)

6. Wash in water for five minutes.

7. Stain in a modified Romanowsky's stain made by mixing just before use—

Azur I., 1 per cent.	1 c.c.
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Eosin, B.A., 1-1000	2 c.c.
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Water	8 c.c.
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for fifteen to thirty minutes.

¹ *Proc. Roy. Soc. Lond.* B. lxxix. p. 102, 1907.

8. Wash.
9. Differentiate in orange-tannin, thirty seconds.
10. Wash well and drain.
11. Absolute alcohol for a few seconds.
12. Alcohol-xylol (in proportion of 2 : 8), two or three changes.
13. Xylol, and mount.

Instead of 7 to 13, any other method of staining can be used, according to what structures it is desired particularly to show.

LEISHMAN-DONOVAN BODY (*Leishmania Donovan*).

The Leishman-Donovan parasite occurs in largenumbers in the spleen and liver, also in the lymphatic glands, lungs,

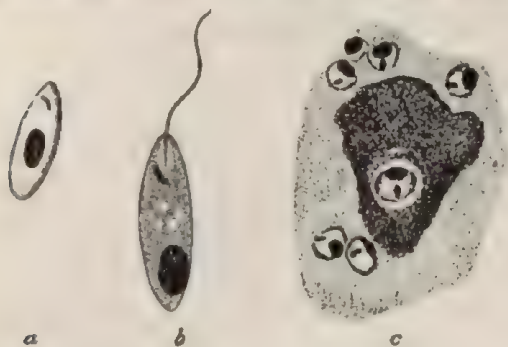


FIG. 57.—*a*, THE LEISHMAN-DONOVAN BODY. *b*, THE FLAGELLATED FORM DEVELOPING IN CITRATED BLOOD. *c*, SEVEN PARASITES IN A LARGE MONONUCLEAR LEUCOCYTE. (After JAMES, PATTON, and ROGERS.)

and intestinal submucosa, and in large mononuclear leucocytes and endothelial cells in Kala-Azar or tropical spleno-megaly. The bodies are small ($2-3\ \mu$), round, ovoid, or oat-shaped masses of protoplasm, apparently encapsuled, and contain two chromatin masses—one large and oval, staining pale red with Leishman's stain; the other small and rod-shaped, and staining deep red with

Leishman (fig. 57, *a*). They sometimes occur in masses (fig. 57, *c*). Leishman considered them to be degenerate trypanosomes. Rogers, however, succeeded in cultivating them in citrated blood at 25° C., in which they develop into flagellated forms like *Herpetomonas* (fig. 57, *b*). The parasite has not been inoculated into animals, and it is probably transmitted to man by a bug.

The bodies are well shown in smears stained with the Leishman stain.

In Oriental sore, or Delhi boil, a parasite practically identical with the Leishman-Donovan body is present, but as the two diseases run a totally different course, it is probably a distinct species.

SPIROCHAETÆ.

The spirochaetes are delicate, undulating, or somewhat spirillar, filiform parasites, occurring in the blood of man, mammals, birds, shell-fish, &c. The filaments taper to a point at the ends, are flexible and motile, coiling and uncoiling, are described as having two nuclear masses, and some possess an undulating membrane, like trypanosomes, but in the smaller forms no definite structure can be made out. They are now generally regarded as protozoa, but some still consider them to be bacteria. Bacterial cells are never pointed, nor do they show the coiling movements of spirochaetes; motility is produced by flagella, which are absent from spirochaetes (statements to the contrary are due to errors of observation and technique), and periodicity is not exhibited by bacteria. Spirochaetes multiply by longitudinal fission, while fission in bacteria is transverse; they react in some cases to drugs (e.g. atoxyl) like trypanosomes, are much more sensitive to the action of immune sera than bacteria are, and are transmitted by insects. No spirochaete has yet been cultivated.

Spirochaete infections may be termed 'spirochaetosis.'

Schaudinn believed that many so-called spirochaetes may be connected with the trypanosomes. In *S. plicatilis* he described the presence of a thread-like nucleus and of chromidia, and of an undulating membrane, but flagella are absent. In the

little owl minute slender trypanosomes occur; these later penetrate leucocytes, and develop into relatively very large trypanosome forms (which have been termed *Leucocytozoa*). These intracorpuseular forms are male and female gametocytes, the male being smaller and more slender than the female. If taken into the gnat's stomach, the male gametocytes give rise to eight microgametes by a process of sporulation, which fertilize the macrogamete, and the resulting zygote ultimately forms by sporulation an immense number of spirochaetes.

In the case of a *Halteridium* parasite of the little owl (*Athene noctua*), Schaudinn claimed to have shown that it is a stage of a trypanosome (*T. noctuæ*) which is disseminated by the common gnat. Minute indifferent, male, or very young female forms being introduced by the gnat's bite into the bird's blood, the male forms soon die out, the indifferent forms multiply and develop into male and female types, and the young female forms grow to maturity. The young forms attach themselves by their flagella to chromocytes, penetrate them, lose their flagella and membranes, and become *Halteridium* forms; after six days the parasite breaks out of the corpuscle and the trypanosome phase once more is assumed, and this alternation recurs again and again, the trypanosome forms multiplying by fission, and the young forms entering the chromocytes. These observations have not been confirmed, and Novy and McNeal believe that Schaudinn was dealing with a double infection of both a trypanosome and a *Halteridium*, not that one was transformed into the other.

Spirochaeta recurrentis (*Obermeieri*).—Found in the blood-plasma, not in the corpuscles, in relapsing fever during the febrile paroxysms. It is very slender and delicate, measuring 12–16 μ in length, and actively motile. It is said to be conveyed by the bed-bug or by pediculi—but this is uncertain—and is inoculable into monkeys, and, less readily, into rats. It has not been cultivated (Plate XX., b).

It is not unlikely that the spirochaetes of relapsing fever in different countries may be distinct species.

Spirochaeta Duttoni.—Found in the blood-plasma in African relapsing, or tick, fever. It closely resembles the *S. recurrentis*, but is more readily inoculable into rats, mice, and guinea-pigs, and the one does not protect against the other. It is conveyed by a tick, *Ornithodoros moubata*.

Blood spirochaetes have been found in many animals, e.g. cattle (*S. Theileri*), mice (*S. muris*), fowls (*S. gallinarum*), and geese (*S. anserina*). Spirochaetes are also present in syphilis (p. 592) and yaws (p. 599), in ulcers, in the mouth (p. 424), and in Vincent's angina (p. 272).

Blood smears may be stained with Leishman's stain.

(On Spirochaetosis, see Nuttall, *Journ. Roy. Inst. Public Health*, xvi, 1908, p. 449.)

TRICHOMONAS VAGINALIS.

This parasite is found in the acid vaginal mucus in 50 per cent. of those examined. It must not be mistaken for a spermatozoon. It is a pear-shaped body, measuring 12 to 30 μ in length, and from the blunt end three flagella are given off.

A much smaller species, *T. intestinalis*, measuring 4 to 15 μ , has been met with in the intestinal canal of man in conditions associated with diarrhoea.

CLASS III.—INFUSORIA (CILLATA).

The Infusoria are protozoa in which locomotive organs consist of cilia and in which the nuclear apparatus is differentiated into a vegetative macronucleus and a generative micronucleus. The cytoplasm is enclosed within a cuticle, an oral aperture is present in the form of a slit or pore, and waste matter is extruded by a pore, constant in position, but, as a rule, visible only when in use. A contractile vacuole is generally present. Reproduction usually takes place by fission which is preceded by division of the two nuclei, the micronucleus by mitosis, the macronucleus by direct division.

The Infusoria are not of much pathological importance, but are common in ponds and ditches, e.g. *Paramecium* and *Vorticella*.

BALANTIDIUM (PARAMECIUM) COLI.

This is an intestinal parasite of swine, occasionally met with in man in conditions associated with chronic diarrhoea and dysentery.

It is somewhat ovoid in shape, the ends being bluntly pointed, is covered with cilia, measures 65 to 85 μ in length, and has a superficial resemblance to the ordinary *Paramecium*.

According to Saville Kent, the *Balantidium coli* is to be distinguished from the ordinary forms of water paramecia by the following characters. The *Bal. coli* is somewhat spindle-shaped or ovoid, and bluntly pointed at each end, one and a half to twice as long as broad, measuring $\frac{3}{8}$ inch to $\frac{1}{8}$ inch in length; the paramecium is more cylindrical, four times as long as broad, measuring $\frac{1}{2}$ inch to $\frac{1}{8}$ inch in length. The oral aperture in *Bal. coli* is near one extremity (fig. 58); in paramecium it is situated at about the middle of the ventral surface. In *Bal. coli* the cilia round the oral aperture are as long again as those over the body generally; in paramecium the whole of the cilia are of the same length.

The *Bal. coli* seems undoubtedly sometimes to be a cause of dysentery.¹



FIG. 59.
BALANTIDIUM COLI.

EXAMINATION OF FLAGELLATED AND CILIATED FORMS.

1. These may be examined fresh in the fluid in which they are present, by mounting on a slide, and covering with a cover-glass, one edge of which rests on a bristle to avoid pressure.

2. Permanent mounts may be made by irrigating with Beale's carmine, washing the stain away with weak glycerin, and mounting in 50 per cent. glycerin.

¹ Strong and Musgrave, *Johns Hopkins Hosp. Bull.* xii., 1901, p. 31; Bureau of Gov. Laboratories, Manila, *Bull.* 26, 1904.

3. Cover-glass preparations may be made in the ordinary way, and the films stained with weak carbol-fuchsin or Leishman's stain. (The organisms are apt to be distorted.)

4. The following method, devised by Rousselet (*Journ. Quekett Microscop. Club*, 2nd Series, vi. No. 36, p. 5, March 1895), for preserving rotatoria may be tried. In those forms which are non-contractile, kill by adding a drop of $\frac{1}{4}$ per cent. osmic acid, wash immediately in water, and preserve in $2\frac{1}{2}$ per cent. formalin. Contractile forms may be first narcotized by adding a drop or two of 2 per cent. cocaine solution, then killed with the osmic and preserved as before.

CLASS IV.—SPOROZOA.

The Sporozoa are exclusively endoparasitic protozoa, the adult without organs of locomotion or for the capture of food, and multiply by some method of sporulation, often very complex. A parasite during the nutritive or 'trophic' phase, when it is absorbing nutriment and growing at the expense of its host, is termed a *trophozoite*; when it is mature and ready for sporulation it is termed a *sporozoite*. The spores are of various kinds and may develop outside the body or in a second host.

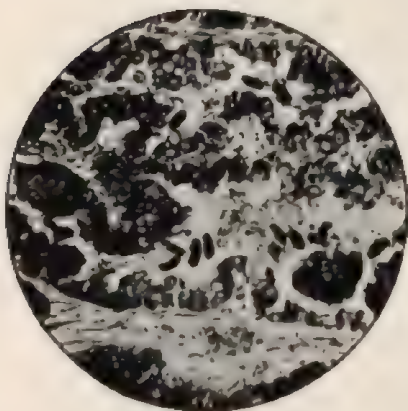
ORDER COCCIDIIDEA.

The Coccidiidea, with a single exception, are intracellular during the trophic stage, and present a dimorphism or alternation of generations; the one is endogenous and asporular, determining the reproduction of the parasite within the host, the other exogenous and sporular and permitting of infection.

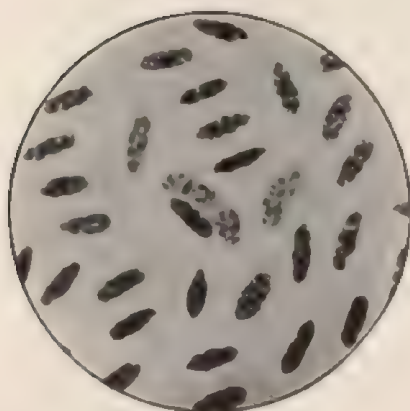
Coccidial Disease of Rabbits.

This is a disease caused by a sporozoon, the *Coccidium oviforme* or *cuniculi*, and often met with in warrens and hutches; in some of the former as many as 90 per cent. of the animals may be affected. The young animals suffer most, and become infected when they cease to suckle and commence to eat green food, the adult animal as a rule resisting the disease. The affected animals waste, suffer from enteritis, and a large proportion

PLATE XXI.



a. *COCCIDIUM OVIFORME*.
SECTION OF RABBIT'S LIVER. $\times 350$.



b. *HALTERIDIUM DANILEWSKYI*.
SMEAR OF PIGEON'S BLOOD. $\times 1500$.



die in from one to three weeks, the condition being known as 'wet-snout' among the keepers. The parasites occur in the intestine, bile ducts, and liver in large numbers. Each parasite is ovoid in shape, measuring $96\ \mu$ in length and $22\ \mu$ in breadth, is enclosed in a firm translucent cyst, which encircles a very granular protoplasm. Sometimes this protoplasm becomes condensed so as to form a spherical mass lying free within the cyst (fig. 59, c). In the intestine and bile ducts the parasites are attached to the epithelial cells, and in the liver, if the animal lives beyond the acute stage, set up some remarkable changes. The affected liver is studded with greyish-white nodules varying



FIG. 59. — *Coccidium oviforme* OF RABBIT: a, *Coccidium* ATTACHED TO AN EPITHELIAL CELL. b-g, STAGES IN THE LIFE-CYCLE. h, FREE SPORES.

in size from a pin's head to a pea. On making sections and examining them microscopically, it is found that these nodules consist of dilated bile ducts filled with a much hypertrophied and convoluted mucous membrane, which forms branched projections covered with cubical epithelium, among which the parasites occur in great numbers (Plate XXI., a). A curious fact is that subcutaneous or intravenous inoculation, or inoculation into the liver of a healthy rabbit with the coccidia from another rabbit, fails to induce the disease.

The coccidium has a complicated life-history, and infection only seems possible in one of the stages. In order to study the

life-cycle the parasite must be placed under suitable conditions, and an infusion of rabbits' faeces, kept at the ordinary temperature, is perhaps as good a cultivating medium as any, the changes being watched by means of interlamellar films. When the coccidia are observed under these conditions, the first change is apparently the formation of micro- and macro-gametes, fusion of these, and the formation of a zygote or oocyst (fig. 59, *b*). The protoplasm of this then condenses so as to form a sphere lying free within the cyst (*c*), a stage sometimes observed in the animal. The sphere then divides into four smaller spherules (*d*). Each spherule becomes elongated, and again divides into two somewhat crescent-shaped bodies, around each pair of which a new, somewhat spindle-shaped capsule forms (*e* and *f*). In this condition the parasite is very resistant, and may remain alive for six months, undergoing no further change unless introduced into another animal. If a young rabbit swallows with its food these crescentic spores, the enclosing capsule is dissolved, and each crescent becomes a rounded amoeboid mass, and this again divides up into many crescentic spores (*g* and *h*). These spores are apparently motile, and enter the epithelial cells of the intestine, gall bladder, and bile ducts, where a process of growth and differentiation occurs, and the fully developed parasite is ultimately reproduced.

Coccidial disease, or, as it is sometimes termed, psorospermiosis, is occasionally met with in animals, as the sheep, and a wasting disease of young pheasants due to coccidia has been described by McFadyean.¹

In man, coccidial disease has been described (but rarely) in the liver, gall bladder, ureter, &c.²

Rixford and Gilchrist³ described two cases of protozoan infection of the skin and organs, accompanied by great destruction of tissue and ending in death. The organisms were spherical, 7 to 27 μ in diameter, surrounded by a thick capsule, enclosing granular bioplasm (*C. immitis*).

The Ruffer-Plimmer bodies of cancer were at one time believed to be coccidia (p. 510).

¹ *Journ. Comp. Path. and Therapeut.* 1895.

² *Journ. Comp. Path. and Bact.* 1898, June, p. 171.

³ *Johns Hopkins Hosp. Reps.* i., 1896, p. 209.

The term 'psorospermiosis' has been applied to human infection with coccidium, *Sarcosporidia* (p. 499), &c.

EXAMINATION.

1. The coccidial forms are readily examined in the fresh state. The only bodies they are likely to be mistaken for are certain ova.

2. Paraffin sections of rabbit's liver containing coccidia may be stained much in the same way as tuberculous tissues—viz. warm carbol-fuchsin ten minutes, decolorize *cautiously* in 5 per cent. acid, and counter-stain in methylene blue. Sections may also be stained in the Ehrlich-Biondi stain for one to two hours.

ORDER HEMOSPORIDIA.

The general characters of this group are :

1. Life at the expense of the red blood-corpuscles, at least during a portion of the life-cycle.

2. Endogenous multiplication by spores, by which the life-cycle is repeated within the host.

3. Development of a form which becomes free in the plasma, and which is the commencement of a sexual cycle to be completed in a second host.

4. Inoculability, but only from one animal to another of the same species.

The group includes the malaria parasite and similar parasites in mammals and birds, the hæmogregarines, *Drepanidium* of the frog, and perhaps the Piroplasmata.

MALARIA.

Malaria is caused by parasitic protozoa, placed in the genus *Plasmodium* (*Hamamaba*), the credit of the discovery of which must be given to Laveran, who described the parasite as occurring in four phases, viz.—(1) spherical bodies, (2) flagellated bodies, (3) crescentic bodies, and (4) segmented or rosette bodies.

The parasites cannot be cultivated, but inoculation of healthy individuals with the blood of malarial patients

reproduces the disease, and the same structures or parasites are found in the blood of these infected persons. Inoculation experiments on all animals except man have proved negative, and in the latter the inoculation must be intravenous.

In the various forms of malarial fever the parasites have the same general characters, though there are distinct differences between them, by which they can be recognized and the type of fever differentiated. In each there is an endo-corporeal cycle within the host, through which the recurrent attacks are developed; there is also an extra-corporeal cycle of development outside the body of the host, whereby the infection of fresh individuals becomes possible. Each of these cycles needs separate description.

If the blood of a malarial patient is examined an hour or two before, or at the very commencement of, the febrile paroxysm, the parasite will be recognized as a pale, ill-defined mass of protoplasm within the red corpuscles, of which a variable proportion are infected, the size of the parasite varying in the different types of fever. When some hours old a variable number of blackish pigment granules of melanin make their appearance. These subsequently coalesce into smaller groups, and the latter again into one or two larger, more or less centrally disposed, masses. The parasites exhibit more or less amœboid movement, and the melanin granules are frequently in a state of tremor. Later on most of the parasites (schizonts) become divided into a variable number of segments, which separate and become spherical, the blood corpuscle breaks down, the spherical bodies or spores are set free, and a certain number of them, again becoming attached to red corpuscles, develop into the first stage of the parasite. The melanin granules and some of the spores are ingested by phagocytes, and the melanin is deposited in the spleen and liver for a time.

The parasite, termed a *plasmodium*, or better an

amabula, contains a vesicular nucleus and a nucleolus, and the melanin granules are present in the surrounding protoplasm. When segmentation occurs, each segment contains a portion of both the nucleolus and the protoplasm. The maturation of each 'brood' of parasites is coincident with a fresh paroxysm. In the sub-tertian forms of malarial fever there exist in the blood for some time after the subsidence of the acute paroxysms well-marked non-motile, crescentic or sausage-shaped bodies, with rounded ends, the so-called 'crescentic bodies' or 'crescents'; their long diameter is greater ($\frac{1}{2}$) than that of a red corpuscle, their protoplasm is finely granular and contains at about the centre several well-marked pigment granules. In the crescentic forms the extremities of the crescent often appear to be joined by a delicate membrane (fig. 65, *f* and *j*); this is the remains of the blood-corpuscle in which the parasite has been developed.

When a 'wet' specimen of malarial blood from a case of pernicious or sub-tertian malaria is kept under observation (p. 492), it not unfrequently happens that after a time the so-called 'flagellated bodies' make their appearance. These consist of a central protoplasmic mass attached to which are from one to six delicate flagella measuring 20-30 μ in length (fig. 60, *c*). The flagella are actively motile and disturb the corpuscles, but the body itself does not move much. Frequently one or more of the flagella break away and swim free, remaining active for several hours. The flagellated bodies are never seen in the freshly drawn blood, and Ross has found that flagellation does not occur if the finger be pricked through a spot of vaseline, the blood remaining covered with the film of grease. Careful observation has shown that the flagellated bodies develop from 'crescents' in sub-tertian malaria, and from rounded parasites, difficult to distinguish from the schizonts, in the benign tertian and quartan fevers.

Various theories were held in the past as to the nature of these flagellated bodies. Through the brilliant researches of ROSS, which have been confirmed and extended by observers in all parts of the world, it is now known that these cells are sexual elements. The flagellated body represents the male cell or 'male gametocyte,' the flagella ('gametes') being analogous to the spermatozoa of higher animals. The female cells or female gametocytes or gametes are non-flagellated, and are fertilized by the entrance of one of the flagella of a male gametocyte. *This*



FIG. 60. DEVELOPMENT OF THE MALARIA PARASITE IN THE MOSQUITO: *a*, *b*, AND *c*, THE MALE GAMETOCYTE; *d*, *e*, AND *f*, THE FEMALE GAMETOCYTE; *f*, FERTILIZATION OF THE FEMALE GAMETOCYTE BY A MICROGAMETE. (After ROSS and FIELDING-QUILL.)

fertilization takes place in the stomach (middle intestine) of certain species of mosquito, and after fertilization a series of changes ensues resulting in the formation of spore-like bodies, which are injected when the insect bites its victim, and thus the infection of fresh individuals with the malaria parasite takes place. The first demonstration of the nature of 'flagellated bodies' was given by Opie and MacCallum on the Halteridium parasite of pigeons, and this forms a good example of the value of abstract research

to practical medicine (see p. 494). Ross also followed the development of the malaria-like 'proteosoma' of sparrows &c. in the mosquito *Culex fatigans*. The development of the malaria parasite of man in the mosquito is as follows, according to Ross and Fielding-Ould.¹ It is not known what determines whether an amœbula will become a sporocyte or a gametocyte. When the sexual cells or 'gametocytes' are ingested with the blood by the mosquito, they pass into the middle intestine. Within a few minutes the corpuscles enclosing them break down, the parasites are set free, and quickly become spherical or ovoid (fig. 60, c, e, and f). One or two spherical granules are often attached to the naked parasites, and may represent polar bodies (fig. 60, c and f). Very soon the male cells become flagellated (fig. 60, c), and before long the flagella or 'microgametes' break away from the parent cell and by their own motility make their way through the liquor sanguinis. Should one come in contact with a female cell or 'macrogamete,' it fuses with the latter, uniting with the nucleus (fig. 60, f), fertilization is completed, and a 'travelling vermicule' or 'ookinet' results; this passes into the outer wall of the mosquito's stomach, where it becomes encysted and forms a 'zygote' (fig. 61, a, b). At this period the zygote is about 7-8 μ in diameter. If development proceeds, it acquires a distinct capsule and begins to grow rapidly, and when mature at the end of a week or more, according to the temperature, is 60 μ in diameter and projects into the body cavity of the insect (fig. 61, b). Its substance next divides into eight to twelve portions, or 'zygotomeres,' then each zygotomere becomes a spherical body, or 'blastophore' (fig. 61, c), and each blastophore develops upon its surface a number of spindle-shaped, radially disposed bodies, or 'zygotoblasts' (fig. 61, d). When the zygote reaches maturity the blastophores

¹ Thompson Yates Laboratories Report, iii. Pt. ii. 1901, p. 183.

disappear, leaving its capsule packed with large numbers ('thousands') of free zygotoblasts. The capsule then ruptures, and the zygotoblasts are poured into the body cavity of the mosquito. The 'blasts' measure $12-16\ \mu$ in length, taper at each extremity, and possess a central nucleus (fig. 61, *e*), and they make their way to all parts

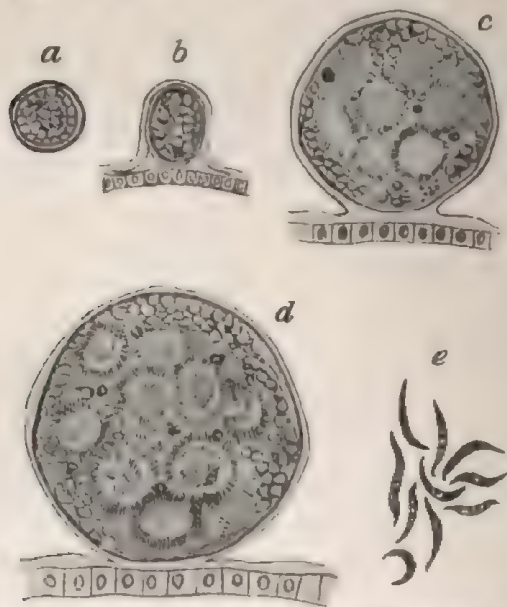


FIG. 61.—DEVELOPMENT OF THE MALARIA PARASITE IN THE MOSQUITO.
(After Ross and FIELDING-OLD.)

of the body of the host, and accumulate in the salivary or poison glands, whence they are discharged by the middle stylet (hypopharynx) of the proboscis, when the insect 'bites,' into the circulation of a fresh vertebrate host. Here, presumably, the blasts become attached to erythrocytes and develop into amœbulae. The diagram¹ (fig. 62)

¹ This figure is reproduced by permission from Daniels' *Laboratory Studies in Tropical Medicine* (Bale, Son & Danielsson, 1908).

represents in graphic form the asexual and sexual cycles of reproduction of the malaria parasite.

So far as is at present known, malarial infection is conveyed only through the bite of infected mosquitoes of the sub-family *Anophelinae*. It has been repeatedly proved that infected mosquitoes convey infection, and that if mosquitoes be excluded human beings may live in the most malarious districts without contracting the disease.

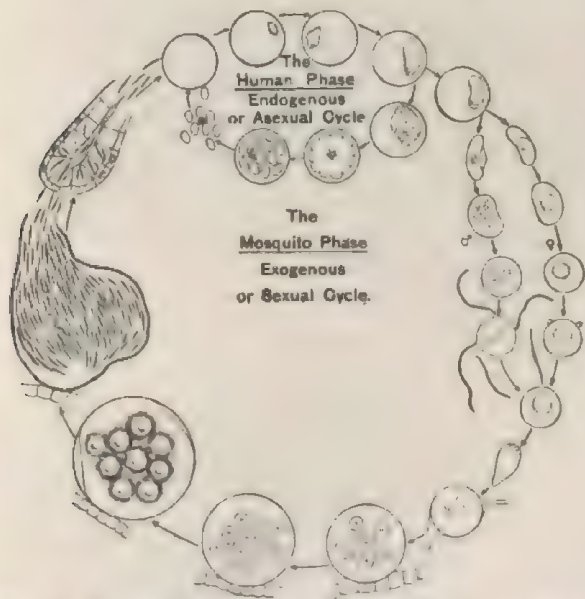


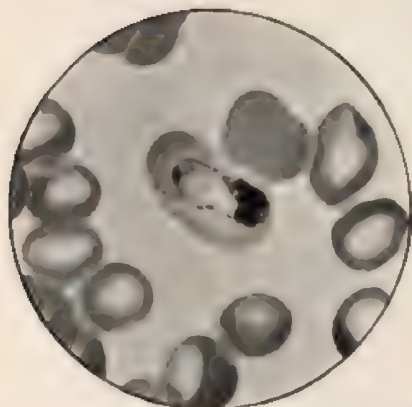
FIG. 62. — DIAGRAM OF THE ASEQUAL AND SEXUAL CYCLES OF THE MALARIA PARASITE.

The common gnats or mosquitoes (*Culicidae*) belong to the genus *Culex* or *Stegomyia*. Those of the *Anophelinae* are usually much less abundant (but there is great variation in different districts), and bite mainly at night. The females alone are blood-suckers. Some species breed in natural collections of stagnant, others in slowly running fresh water well supplied with

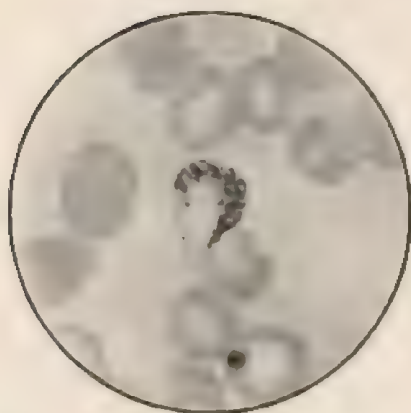
MANUAL OF BACTERIOLOGY

ns of vegetable life. If the head of a mosquito be with a hand-lens, three sets of appendages will be in the middle is the stout proboscis containing the and suctorial apparatus; situated at the base of this are one on either side, and outside these again are two which are more or less hairy. In *Anophelineæ*, both female, the palpi are as long as the proboscis; in the *Culex* (also in *Stegomyia* and many other genera) they are short and stumpy. In *Anophelineæ* the scales on the veins are usually arranged in alternating light and dark giving a speckled or dappled appearance, different as anything seen in *Culex*. (Some *Culices* have a similar pattern, and it is wanting in *A. maculipennis* and *bifurcatus*.) The outer costal margin of the wing in *Anophelineæ* is almost always marked with dark blotches. *Anopheles*, as a whole, is a slender insect than *Culex*, and when at rest its body is all straight, whereas *Culex* is angular or humpbacked. The species known to carry malaria are *Anopheles maculipennis* in Europe, N. Africa, and N. America, *A. bifurcatus* in India, *Stegomyia funesta* and *Pyretophorus costalis* in Central

PLATE XXII.



a. MALARIA. PARASITE OF BENIGN TERTIAN FEVER.
SMEAR OF BLOOD. $\times 1500$.



b. MALARIA. GAMETOCYTE OF BENIGN TERTIAN PARASITE
SMEAR OF BLOOD. $\times 1500$.

[REDACTED]

without an attack, and reckoning the day of the previous attack, an attack occurs every fourth day, hence the name 'quartan.' It commences as a small amebula which is feebly motile. It enlarges, becomes pigmented, and motility ceases, the pigment granules being numerous and coarse. The parasite finally occupies nearly the whole of the corpuscle, which, however, is but little altered.

Towards the end of the apyrexial period the pigment collects in the centre and segmentation takes place with the formation of a symmetrical rosette (*e*) and afterwards of six to twelve spores (*f*). The quartan parasite does not form



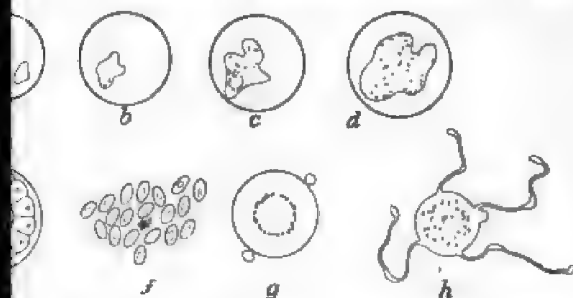
FIG. 63.—THE QUARTAN PARASITE: *a*, *b*, *c*, *d*, AMEBULA; *e*, NEOBOCYTE; *f*, FREE SPORES; *g*, FEMALE GAMETOCYTE WITH SO-CALLED POLAR BODY; *h*, MALE GAMETOCYTE. (After Rees.)

crescents, and the flagellated bodies (*h*), which are rarely seen, are developed from large pigmented parasites.

2. *Benign, or Spring, Tertian Fever* (fig. 64, Plate XXII., *a*).—The benign tertian parasite (*Plasmodium vivax*) completes its asexual life cycle in forty-eight hours, an attack occurring every other day, or, reckoning the day of the previous attack, every third day. In the early stage it resembles the quartan, but shows much more active amoeboid movement. The pigment granules are also finer than in the quartan, and incessantly change their position. The parasite finally invades the whole corpuscle, which becomes

MANUAL OF BACTERIOLOGY

and pale. Enlargement of the corpuscles is a feature in the benign tertian infection. Maturation takes place, but is unsymmetrical (*e*), in the formation of a grape-like cluster of twelve



THE BENIGN TERTIAN PARASITE: *a, b, c, d*. AMEBULE; *e*. FREE SPORE; *f*. FREE SPORES; *g*. FEMALE GAMETOCYTE WITH SO-CALLED EDGES; *h*. MALE GAMETOCYTE. (After REES.)

spores (*f*). As in the quartan, no crescentic forms are developed, and the gametocytes (*g, h*) are similar

the unpigmented quotidian and the malignant tertian, but this is not generally accepted) is much smaller than the quartan or benign tertian, and when it reaches the stage of multiplication it disappears from the peripheral blood and collects in the internal organs, spleen, liver, cerebral capillaries, and bone-marrow. It is actively amœboid, seems to change its position within the corpuscle, and the pigment granules are very fine in the young parasites, but early aggregate into large clumps. The fission forms (*d, e*) are only met with in the internal organs. Multiple infection of the corpuscles may also occur. The corpuscles often suffer severely from the infection, some being shrivelled and spinous, others dark in colour, 'brassy'; they may also be altered or destroyed without being actually invaded by the parasite. It is in this form that the crescentic bodies appear (*f, j*). These, however, are not met with at the very commencement of the attack, but appear in a week or so, and may not disappear until some weeks after the termination of the attack. This parasite is met with in the sub-tertian, or so-called malignant, types of fever, which are characterized by irregularity of the fever, considerable blood destruction, often accompanied by hæmoglobinuria, and cachexia; coma is another complication in certain instances, probably caused by massing of the parasites in the cerebral capillaries.

The cure of malaria by quinine is regarded as being due to a poisonous action on the parasites analogous to that exerted on numerous protozoa; amœbæ, for example, being injuriously affected by so little as a 1-50,000 solution of quinine hydrochlorate.

No toxin can usually be demonstrated in the blood of those suffering from a malarial attack, but Rosenau and his co-workers have found that the filtered blood, *taken when the temperature is rising*, produces a malaria-like paroxysm.¹

¹ See Hewlett, *loc. cit.* p. 144.

A malaria-like parasite (*Plas. Kochi*) occurs in apes, in which it produces fever.

The nature of Blackwater fever, so called from the presence of hæmaturia and hæmoglobinuria, has given rise to much discussion. By some it is considered to be a disease *sui generis*, of unknown ætiology. By others it is regarded as a form of malaria, either of an intense type, or in which the kidneys are especially involved, or as due to malarial infection *plus* quinine. It may be that under particular conditions, of the nature of which we are at present ignorant, hæmolysins may be set free and cause hæmolysis, the blood pigment being eliminated by the kidneys.¹

CLINICAL EXAMINATION.

The blood of malarial patients may be examined either in the unstained or stained condition.

Examination in the unstained condition.—The finger or lobe of the ear is pricked, and a droplet of blood taken up on a clean cover-glass, which is then placed upon a slide, so that the droplet of blood spreads out into a thin layer between the two glasses. The cover-glass may then be ringed with oil or vaseline to prevent evaporation. A little practice is required to judge the right quantity of blood. The preparation should be examined with a $\frac{1}{8}$ -inch oil-immersion lens.

Examination in the stained condition. To prepare stained specimens the finger or ear is pricked as before, and a droplet of blood taken up on a cover-glass; another cover-glass is applied, and the two are separated so that each is smeared with a thin film of blood; several are prepared in this manner. Manson recommends picking up a droplet of blood on an oblong slip of fine clean tissue or cigarette paper. The charged surface of the paper is then applied to a clean glass slide: in a second or so the blood will have formed a thin film between the slide and the tissue paper. The latter is then withdrawn, leaving a very thin film on the glass, and may be applied to a second slide, and, in like manner, to three or four in succession. A piece of gutta-percha tissue may be similarly used. Or a droplet of blood may be picked up on a slide near one end, and the edge of a second

¹ See Hewlett, *loc. cit.* p. 145.

slide held at an angle of 45° being applied to it, the blood is spread by *pushing* the second slide over the first one, or the droplet of blood on a slide may be spread by touching it with a needle held flat on the slide and drawn evenly along the surface of the slide. Whatever method is adopted, the film is allowed to dry in the air, and may then be fixed (not if Leishman's stain is used) by heat, preferably at 110° C. for one hour, as overheating ruins the preparations. It is much simpler and better to fix in a mixture of equal parts of absolute alcohol and ether for not less than ten minutes, preferably for half an hour; this gives excellent results. In hot countries a saturated solution of corrosive sublimate may be used. The methods detailed at p. 87 may also be employed.

As regards staining, this is usually carried out with Leishman's stain (No. 13, p. 92). The blood films, *unfixed*, are flooded with a few drops (5-10) of the stain, which is spread by tilting, no attempt being made to check evaporation. After half a minute about double the quantity of distilled water is added, allowed to mix with the stain on the film, and staining is continued for five or in some cases for ten minutes. The film is then washed in distilled water, some of the water allowed to remain on the film for one minute, and it is then dried and mounted. Jenner's blood stain may be similarly used.

Staining may also be done in a half-saturated aqueous solution of methylene blue or in Löffler's blue for half an hour, washing in water, and counter-staining with a very weak eosin solution for a few seconds, washing, drying, and mounting. Manson recommends treating the films with very weak acetic acid—2 or 3 drops to the ounce of water—to wash out the hæmoglobin, and, after washing, staining in the following solution for half a minute:

Borax	5 parts
Methylene blue	0.5 part
Water	100 parts

washing, drying, and mounting in xylol balsam.

Hæmatoxylin (Ehrlich's, or Mayer's hæmalum) is preferable for permanent preparations, and in hot countries, where methylene

blue rapidly fades. The preparations may be counter-stained with a weak solution of eosin.

Ross recommends for rapid diagnosis the use of *thick* blood films, from which the hæmoglobin is first removed with very dilute acetic acid; the films are then stained with Leishman's stain, and examined with a $\frac{1}{8}$ -inch objective. Practice is required for this method.

In order to demonstrate the flagellated organisms Manson recommends the following procedure: Thirty or forty strips of thick blotting-paper (8 inches by $1\frac{1}{2}$ inch), each having an oblong hole ($\frac{3}{4}$ inch by $\frac{5}{8}$ inch) cut lengthways in the centre, are prepared, moistened with water, and laid on a sheet of window-glass. A patient is selected in whose blood the crescentic form is plentiful, and a minute droplet of the blood, about the size of a pin's head, is expressed from a prick. A clean slide is then breathed on, and the droplet of blood picked up on it and spread out with a needle so as to cover an area $\frac{3}{4}$ inch by $\frac{1}{2}$ inch. The slide is immediately inverted over a blotting-paper cell and pressed down sufficiently to secure perfect apposition. The rest of the paper cells are similarly covered with blood-charged slides. In from half to three-quarters of an hour the slides are removed and dried by gentle warming, and then fixed with absolute alcohol for five minutes. The alcohol is allowed to evaporate, and the films are treated with a few drops of 15 per cent. acetic acid to dissolve out the hæmoglobin. The slides are then washed in water and stained with weak carbol fuchsin (20 per cent.) for six to eight hours, washed in water, dried, and mounted.

N.B.—Negative results in the examination for the malaria parasite must be accepted with caution unless repeated. A single undoubted parasite is sufficient to establish the diagnosis. Quinine causes the disappearance of the parasite. The parasites in the sub-tertian fever disappear during the apyrexial intervals (except the crescents), and are most likely to be found at the commencement of the attack i.e. when the temperature is rising. The parasites of the other forms are larger and more obvious during the apyrexial intervals.

[For further particulars on Malaria and on the demonstration of the malaria parasite, see Daniels' *Laboratory Studies in Tropical Medicine*, 1908.]

PLASMODIUM PRÆCOX.

Syn. *Proteosoma Grassii*, *Hæmamarba relicta*.

This parasite (commonly called 'proteosoma') is met with in sparrows and other birds, in which it invades the red blood-corpuscles, and has many similarities with the malarial parasite of man. In its youngest stage it occurs as a round, clear, refractive, non-amoeboid granule, not unlike a vacuole. As the organism grows pigment granules appear; these alter the position they occupy from time to time, it becomes irregular in shape, and probably slow amoeboid movement occurs, though this is not observable. At first situated in any part of the corpuscle, it finally comes to occupy one or other extremity, and as it enlarges the nucleus of the corpuscle becomes dislocated. Lastly, the parasite assumes an approximately spherical shape, the pigment collects in the centre, segmentation occurs, and a 'rosette' forms; it then divides into five to twenty portions, all of which become spherical. The corpuscle disintegrates, these bodies or 'spores' are set free, probably again become attached to fresh corpuscles, and the cycle is repeated. In some specimens of blood flagellated male gametocytes make their appearance, similar to those of malaria, the flagella break away from the main mass, fertilize other non-flagellated or female cells, and a series of changes ensues analogous to those occurring in the malarial parasite (p. 483). The fertilization and development of the fertilized cell take place in the stomach of a mosquito (*Culex fatigans*) which has sucked the blood of a bird containing the parasites, and subsequently, when this mosquito bites an uninfected bird, the infection is transmitted.

HALTERIDIUM DANILEWSKYI.

This is an elongated, curved parasite (also known as *Hæmoproteus* or *Hæmamarba Danilewskyi*) found in the red corpuscles of certain birds (pigeon, crow, &c.), and embracing the nucleus (Plate XXI., b). By some it is included among the malaria-like parasites (*Plasmodium*). At an early stage it much resembles the proteosoma, but as it grows it becomes elongated, pigment granules appear, and are either scattered throughout the

protoplasm or collect in two groups, one at each extremity. Finally, the parasite occupies nearly the whole of the corpuscle, dislocating its nucleus. The fully grown parasites may be differentiated into two forms, one of which remains almost completely unstained when treated with methylene blue, the other staining deeply with this dye (Opie). When the blood is withdrawn, the corpuscles disintegrate and liberate the contained parasites, which assume a circular outline, and a certain number become flagellated. *It is only the non-staining form which becomes flagellated.* These two varieties of the parasite are the male and female cells respectively, and the fertilization of the female cell by a free flagellum has been actually observed by MacCallum.¹ It can hardly be doubted that the development of the fertilized cells takes place in some insect, but the definitive host has not yet been discovered with certainty.

The presence of these parasites induces rise of temperature, deposition of melanin, and changes in, and enlargement of, the spleen and liver analogous to those occurring in malaria in man. The *Halteridium* parasite, according to Schaudinn, is a stage in the life-cycle of a trypanosome (see p. 478).

THE PIROPLASMATA.²

Syn. Pyrosoma, Babesia.

The Piroplasmata form a somewhat anomalous group, but are usually included in the Haemosporidia of the Sporozoa. They differ from the *Plasmodia* by not possessing pigment, by the nucleolus not fragmenting, by division into two or four only, and by the frequency of extra-corpuscular forms. They cause many diseases in animals, are conveyed by ticks, but are unknown in man. (A piroplasma was described as the causative organism of Rocky Mountain spotted fever by Wilson and Chowning, but the observations appear to be erroneous.) The body of a piroplasma is typically pear-shaped, but rounded and rod forms occur. Two nuclear masses are present, one larger than the other.

¹ *Journ. Exper. Med.* iii., 1898, pp. 79, 103, 117.

² See Hewlett, *Trans. XIV. Internat. Cong. of Hygiene*, Berlin, ii., 1908, p. 146; Minchin in *Allbutt's System of Med.* Ed. 2, vol. ii. Pt. 2, p. 86.

The developmental cycle in the ticks has not been worked out, but Koch has observed peculiar rayed forms with *P. bigeminum*, and Christopher's¹ various developmental forms with *P. canis*. Miyajima states that a piroplasma of Japanese cattle (apparently *P. parvum*) in blood broth develops into typical trypanosome forms.²

Piroplasma bigeminum. This is the parasite of the well-known Texas fever of cattle, a disease which is characterized by fever, emaciation, anæmia, hemoglobinuria, and enlargement of the liver and spleen.

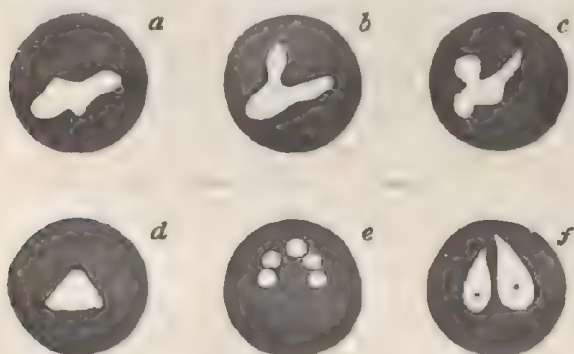


FIG. 66.—*PIROPLASMA BIGEMINUM*. (After CELLI.)

The disease causes considerable loss among cattle, and is met with in various parts of the world, America, Australia, South Africa, Malaya, the Philippines, the Roman Campagna, Greece, Roumania, and North Ireland.

In the acute type of the disease a small proportion (1-5 per cent.) of the red corpuscles in the peripheral circulation contain pairs of pyriform bodies 2-4 μ in length and 1.5-2 μ in largest diameter. One end of each body is rounded, and the body gradually tapers to a point at the other end, and the pair lie close together, their tapering ends directed towards each other. A dark spherical body is present at the rounded end of the parasite (fig. 66, f).

¹ Brit. Med. Journ. 1907, i. p. 76.

² Philippine Journ. of Science, ii., 1907, p. 83.

Some of the young parasites exhibit amœboid movements when the blood is examined on a warm stage (fig. 66, *a, b, c*). In the internal organs the parasites are more numerous; in the kidney and liver 10-25 per cent. of the corpuscles contain them, in the heart-muscle 50 per cent. In the mild type 5-50 per cent. of the corpuscles in the circulating blood may be infected at one time or another, and the parasite appears in some cases as a coccus-like body at the periphery of the corpuscle. This appears to become enlarged and spindle-shaped, then to taper in the middle, divide, and so give rise to the pyriform bodies. Occasionally minute free cœcoid bodies are seen in the plasma, and at times two to five minute (0.5 μ) cœcoid cells are present in the red cells (fig. 66, *e*). After death the pyriform bodies seem to become spherical or angular (fig. 66, *d*).

Sexually differentiated gametes are not known with certainty, but flagellated forms have been described.

The disease is transmitted through the bites of ticks (*Rhipicephalus annulatus*, *R. australis*). The female tick, after biting an infected ox and sucking its blood, falls off and lays its eggs; the eggs hatch in two to six weeks' time, and the daughter ticks transmit the disease to other animals through their bites.¹ The disease may be to some extent controlled by prophylactic measures designed to destroy the ticks, and to prevent infection thereby.

A partial immunity is enjoyed after an attack of the disease, but by repeated attacks the immunity may be rendered absolute. By inoculation with the blood of an affected animal in which the fever has subsided, a transient illness in the inoculated animal is produced together with partial immunity, and by a second or third inoculation the immunity may be much increased. The mortality from such a procedure amounts to 3-5 per cent.²

P. parvum causes Rhodesian red-water of cattle. It is not directly inoculable, and is conveyed by the tick *R. appendiculatus*.

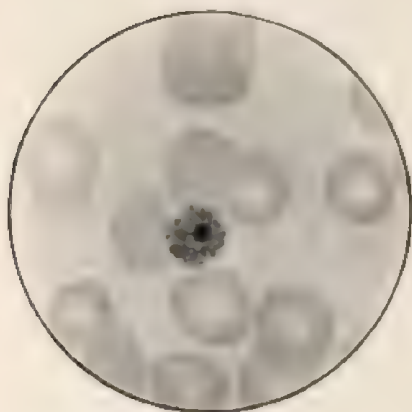
P. equi causes biliary fever in horses.

P. canis causes epidemic jaundice in dogs (Plate XXIII., *b*). It is conveyed by the ticks *Hæmaphysalis leachi* in South Africa,

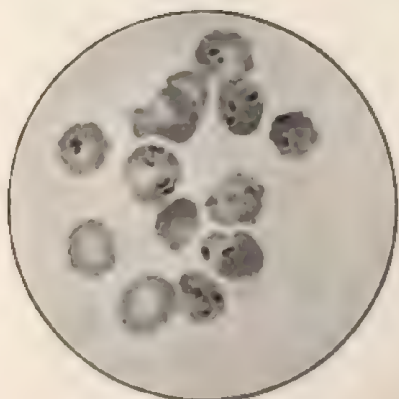
¹ See Smith and Kilborne, *Texas or Southern Cattle Fever*, United States Dep. Agricult. Bull. No. 1, 1893.

² See Tidswell, *Report on Protective Inoculation against Tick Fever*, New South Wales, Dep. Pub. Health, i. 1898; ii. 1900.

PLATE XXIII.



a. MALARIA. A TERTIAN 'ROSETTE'.
SMEAR OF BLOOD. $\times 1500$.



b. PIROPLASMA CANIS.
SMEAR OF BLOOD. $\times 1500$

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R. sanguineus in India, and *Dermacentor reticulatus* in Europe.¹ (On Ticks, see Nuttall, *Journ. Roy. Inst. of Public Health*, xvi., 1908, p. 385.)

HÆMOGREGARINA.

The hæmogregarines are unpigmented parasites, not amœboid, typically having an elongated body or vermicule, occurring in the blood, mostly in cold-blooded vertebrates, but several species have of late been found in mammals (dog, jerboa, palm squirrel), though not in man. In the dog, the parasite (*Leucocytozoon*

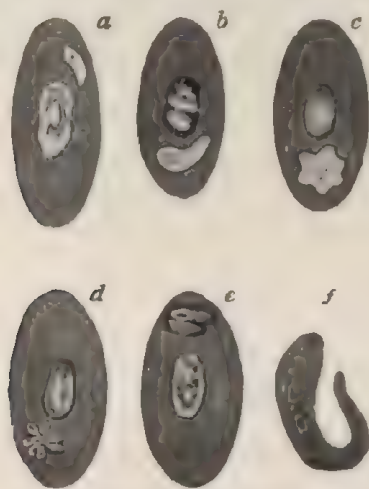


FIG. 67.—*DREPANIDIUM RANARUM*. (After CELLÉ.)

cantis) occurs as an elongated, curved or doubled-up, body in the polymorphonuclear leucocytes. It is encapsuled and contains a single granular nucleus. Encystment with sporulation occurs in the bone-marrow, and a sexual development is stated to occur in a tick.

Hæmogregarina (*Drepanidium*, *Lankesterella*) *ranarum* inhabits frogs (*Rana esculenta*) and possesses both an intra- and an extra-corporeal phase. In the former the parasite commences as an elongated gregarine-like body (fig. 67, a and b) which

¹ See Nuttall and Graham-Smith, *Journ. of Hygiene*, iv.-viii., 1904-08.

MANUAL OF BACTERIOLOGY

in size until its length is $10-15\ \mu$; it then divides into small or a few large gymnosporos. In the first case they may number fifty, are $3-5\ \mu$ in length, occur in May or June, and are exclusively within the erythrocytes; in the latter case the spores measure $5-8\ \mu$ in length, are five to fifteen in number (fig. 67, *c* and *d*), and develop within cells in the lymphatic organs. The extra-corpuscular phase, commencing with the formation of the corpuscles, ends in an elongated organism possessing ciliary movement, and free in the plasma (*f*). The parasite occurs not only in the blood, but also in the cells of the liver, and bone-marrow, and has also been met with in the intestinal tract. By transfusion of infected blood the parasite can be conveyed from one frog to another, but the mode of transmission in nature and in what definitive host its life is completed are unknown.

Johnson and Myers investigated the life-history of a parasite found in a Brazilian toad, and believe that the parasite is passed in the body of a tick infesting the toads.

By allowing each moth to lay its eggs separately, and subsequently examining the body of the moth microscopically, he was able to separate the healthy from the diseased, and the eggs of the former were kept, while those of the latter were destroyed. According to Pfeiffer,¹ when the worms eat the excreta containing the corpuscles mentioned above, these lose their capsule and form large amœboid masses which penetrate the muscles and blood-corpuscles. The amœboid masses then become encapsuled, and are yellow and granular. Later on the bright roundish corpuscles form within them.

Another disease of silkworms is known as flacherie, but is due to a bacterium, *Micrococcus bombycis*. It is contagious, and can be transmitted by inoculation.

ORDER SARCOSPORIDIA.

The parasites belonging to this order are not thoroughly worked out. They complete their life-history in the substance of striated muscular fibres: such are the well-known Miescher's corpuscles. Few instances of this class of parasite are recorded in man, but it occurs in the monkey.² T. Smith³ describes the characters and development of a species found in mice.

ADDENDUM.

With regard to the transmission of trypanosomiasis by tsetse flies, Roubaud has observed that immediately after the fly has fed on infected blood, its proboscis contains actively motile trypanosomes, which soon change into herpetomous forms and the parasites undergo considerable multiplication. Roubaud regards this development as a temporary culture, and the power of multiplying was found to be a specific relation between the trypanosomes and the tsetse. These observations suggest that pathogenic trypanosomes possess a specific power of adapting themselves to the salivary secretion of the tsetse, and thus explain the peculiar relation between these flies and the spread by them of trypanosome diseases.⁴

¹ *Zeitschr. f. Hyg.* iii., 1888, p. 3.

² De Korte, *Journ. of Hygiene*, v., 1905, p. 451.

³ *Journ. Exper. Med.* vi. No. 1, 1901, p. 1.

⁴ *Nature*, 1908, August 20, p. 572.

MANUAL OF BACTERIOLOGY

CHAPTER XIX.

DISEASES OF UNCERTAIN ETIOLOGY.

Fever—Hydrophobia—Malignant Disease—Vaccinia and Variola.

SCARLET FEVER.

Various organisms have been described in scarlet fever—
by Eddington, a streptococcus by Frankel and
berg, protozoa by Mallory and others. The disease
milk-borne, and in the historic Hendon outbreak, a
occus was claimed by Klein to be the specific infec-
ant, but the researches of Crookshank and others

which Klein and Power came to were, therefore, that scarlet fever is communicable to, and may exist in, cows, the milk thereby becoming infected and conveying the disease to man, and that a streptococcus is the specific infective agent.

The Hendon outbreak was reinvestigated by Axe and Crookshank.¹ Axe found that, so far from there being no source of human infection, cases of scarlet fever had occurred near the dairy within a short time of the outbreak, and the eruptive disease of the cow was shown by Crookshank to be cow-pox, while the so-called streptococcus of scarlet fever he regarded as a variety of the *S. pyogenes*. The existence of bovine scarlet fever is entirely discredited by the veterinary profession, both here and on the Continent.

Gordon² reinvestigated the bacteriology of scarlatina with special reference to the *Streptococcus scarlatinae* or *conglomeratus* of Klein. He found that this organism differs distinctly in its cultural characters from other varieties of streptococci, and that it occurs constantly in the mucous secretion on the surface of the tonsils and fauces and in the nasal, but not in the aural, discharge in scarlatina. It is also present in a somewhat modified form in the blood and tissues post mortem. It was not found in four non-scarlatinal throats examined. Gordon concluded therefore that the *S. scarlatinae* or *conglomeratus* is the 'specialized and essential agent' of scarlatina. It is pathogenic to mice.

Cumpston¹ has investigated the biological characters of 101 streptococci isolated from scarlet fever, applying Gordon's tests (p. 213). The majority correspond with the *S. longus* type.

¹ On the Hendon outbreak, see *Trans. Path. Soc. Lond.* 1888 (Refs.).

² (a) *Rep. Med. Off. Loc. Gov. Board* for 1898-99, p. 480; (b) *ibid.* for 1899-1900, p. 385.

³ *Journ. of Hyg.* vii., 1907, p. 599.

MANUAL OF BACTERIOLOGY

nsky and Sommerfeld¹ also isolated a streptococcus *latina*, but were unable to find that it had any cultural characters.

likewise isolated a very similar organism (*Diplo-arlatae*). He claims that it is probably identical t described by Baginsky and Sommerfeld. The medium used was glycerin agar with 5 per cent. of arth.

es² investigated the characters of the organism of He describes it as appearing either as a micrococcus, us, streptococcus, or bacterium. On Löffler's blood forms a whitish sticky growth.

ems very doubtful if streptococci are the aetiological a scarlet fever; they are probably secondary infec- its. It is remarkable how frequently diphtheria tes scarlatina.

ry has detected small bodies, 2-7 μ in diameter, delicately but sharply with methylene blue, and

inoculation. The disease may assume two forms, either the raging or the paralytic. The latter is not met with in man, unless certain rare forms of acute ascending paralysis (e.g. Landry's) be manifestations of it. In the dog either may occur, but in rodents the paralytic form is almost always the one assumed. In man the incubation period is very variable; it is never less than about twenty days, and possibly may be as long as two years, or even more; the average seems to be about ten weeks. In the rabbit, after inoculation from the dog, the incubation period is about two to three weeks.

The virus resides in the central nervous system, as was shown by Pasteur. Inoculation with emulsions prepared from the medulla and with the saliva conveys the disease, but the *filtered* emulsions are usually inactive, and the other tissues and fluids of the body, excepting the lachrymals and suprarenals, are non-infective.

Remlinger¹ has found that after very complete trituration the virus may pass through a porcelain filter.

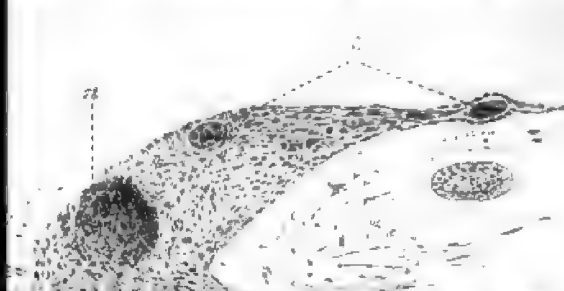
No micro-organism has been demonstrated with certainty in rabies. Negri has described the constant presence of structures, the Negri bodies, particularly in the grey matter of the hippocampus major, which he regards as protozoa. They are of varying size, apparently encapsuled, taking a homogeneous purplish colour in smears stained with eosin and methylene blue, the smallest spherical and structureless, larger ones with a central granule or nucleus, the largest, round, ovoid or elongated, containing several (as many as eight) granules (fig. 68). They occur abundantly in animals suffering from chronic rabies, but in the acute type are scanty, though still to be found; in 'fixed virus' (p. 505) they are very small. So constantly are the Negri bodies present in rabies, and absent in non-rabic

¹ *Bull. de l'Inst. Pasteur*, iv., 1904 p. 342.

MANUAL OF BACTERIOLOGY

ns, that their presence or absence forms a rapid
ple means of diagnosis.¹

s² states that the virus is destroyed at a tempera-
60° C., but the medulla and other infective material
their virulence for months in glycerin. He has
l certain lesions present in the medulla in cases of
he so-called rabic tubercles. These consist of an
of the peri-ganglionic spaces by an accumulation
cells, with degeneration of the cells of the bulbar



ganglionic cells, which become shrunken and no longer fill the enclosing capsule, and their nuclei at the same time become ill-defined and stain badly. A number of new-formed cells also appear within the ganglionic capsules. Ravenel and McCarthy studied twenty-eight cases of rabies in various animals, and consider that these capsular and cellular changes in the ganglia, taken in conjunction with the clinical manifestations, afford a rapid and trustworthy means of diagnosis of rabies, but that the absence of these changes does not necessarily imply that rabies is not present. They also consider that the rabic tubercle of Babes is present sufficiently often to furnish valuable assistance in cases where the central nervous system only is obtainable.¹

Pasteur showed that the virus could be attenuated by desiccating the infective nerve matter, and in this way was able to prepare a vaccine which would protect animals from otherwise fatal doses of the virus. Advancing a step further, he used his vaccines to treat individuals who had been bitten by rabid animals, but in whom the symptoms had not yet developed, and so inaugurated the present system of anti-rabic inoculation as carried out at the Pasteur and other Institutes.

To prepare the anti-rabic vaccines a rabbit is inoculated subdurally with an emulsion made from the medulla of a rabid dog. When the animal dies a second rabbit is similarly inoculated from the first, and the passage through rabbits is continued until a 'fixed' virus is obtained, with which the first symptoms appear on the seventh or eighth day, and which kills with certainty in about ten days. This having been attained, two or three rabbits are inoculated subdurally every day, so that there is a daily supply of animals dead of the disease. The spinal cord is removed

¹ See *Journ. Compar. Pathol. and Therapeut.* xiv. 14. i., 1901, p. 37.

MANUAL OF BACTERIOLOGY

aseptic precautions, cut into convenient segments, and placed in bell-jars containing a layer of caustic potash on the bottom, which serves to desiccate them. The jars are sealed and preserved in glass cases in a dark room, at a constant temperature of about 23° C. In Paris the vaccine fluids are prepared by triturating portions of the cords in sterile broth, so as to form an emulsion. 1 cm. of cord in 5 c.c. of sterile broth, of which 0.5 c.c. (2 mm. of cord) forms a single dose. At the commencement of treatment the cords which have been used for fourteen days are used, at the end of treatment the cords which have been dried for only three days; the latter are more virulent, and would communicate the disease for the previous treatment. The rabbits employed must all be of the same weight (2½ kilogrammes in Paris); if the rabbits are small a slightly shorter period of desiccation of the cords would be necessary. The treatment is continued according to the severity of the case, and is terminated by the removal and situation of the bitac

employed at Lille, 2 mm. of cord being emulsified in 5 c.c. of sterile broth or physiological salt solution :

ORDINARY TREATMENT.		ORDINARY TREATMENT.	
Day of Treatment.	Days of Desiccation of Cord.	Day of Treatment.	Days of Desiccation of Cord.
1 (two injections)	14 and 13	13	3
2	12 and 11	14 (two injections)	9 and 8
3	10 and 9	15	7 and 6
4	8 and 7	16	5
5	6	17	4
6	5	18	3
7	4		
8	3		
9 (two injections)	9 and 8	FOR SEVERE BITES, in Addition.	
10	7 and 6	19 (two injections)	7 and 6
11	5	20	5 and 4
12	4	21	3

At Buda-Pest a dilution method has been employed ; instead of drying the cords, an emulsion is made with the fresh cord, and this emulsion is considerably diluted for the earlier doses, dilutions of 1 in 10,000 to 1 in 6000 corresponding to cords dried for from fourteen to eight days.¹

Undoubtedly the Pasteur inoculations will protect animals from rabies, the duration of immunity after vaccination in the dog being at least three years. In man the efficacy of the treatment can only be judged by statistics. The mortality after bites by supposed rabid animals is variously stated, the most favourable being about 16 per cent. (Leblanc). At the Pasteur Institute, Paris, among 2730 cases treated in which the animal which inflicted the bites was proved to be rabid by inoculation experiments, nineteen deaths occurred, a mortality of 0·7 per cent. In 1905, 727 cases were treated, with 3 deaths ; in 1906, 772 cases, with 1 death ; in 1907, 786 cases, with 3 deaths, being mortalities of 0·41, 0·13, and 0·38 per cent. respectively.

The failure of the treatment may be due to two causes : (1) delay in its commencement, and (2) a short incubation

¹ See Cabot, *Journ. Exp. Med.* iv., 1899, p. 182.

period. The principle of the treatment probably depends upon the long incubation period of the disease, owing to which it is possible to forestall the disease and to immunize the body by the inoculations before its onset. If, unfortunately, the infective material should be very virulent, and the incubation period thereby reduced to the lower limit, it may be impossible to do this before the onset of the disease, and the same is the case if the commencement of the treatment be delayed. Pasteur's system of inoculation is useless when the disease has declared itself.

By vaccinating animals by the Pasteur method by a long series of injections, and with the most virulent material, the blood serum acquires 'anti-' properties, and this 'anti-rabic' serum is said to be of service in the treatment of the declared disease.

DIAGNOSIS OF RABIES.

In a case of suspected rabies in a dog, the animal should *not* be killed immediately, but should be kept under observation for three or four weeks and then killed.

1. Moderately thin smears on slides are made from (a) the cortex in the region of the fissure of Rolando (the crucial sulcus in the dog), (b) the hippocampus major, (c) the cerebellum. They are dried in the air, fixed for five minutes in methyl alcohol, and then stained in weak Giemsa (1 drop stain, 1 c.c. distilled water ; with 1 drop of 1 per cent. potassium carbonate solution to every 10 c.c. of the dilute stain) for three hours. The stained films are then washed in running tap-water for one to three minutes, dried with filter-paper, and examined for the Negri bodies.

Or the moist films may be fixed in methyl alcohol, and without drying stained for one minute in a mixture of 10 c.c. distilled water, 3 drops of a saturated alcoholic solution of basic fuchsin, and 2 c.c. of Löffler's methylene blue. Ecsin-methylene blue mixtures may also be used.

The cytoplasm of the bodies stains orange, pink, red, or magenta, the central nuclei are granular and appear bluish or purplish.

2. If the Negri bodies cannot be detected, inoculation should be performed. The brain should be removed as soon as possible, and if it cannot be manipulated immediately, should be placed in sterile glycerin. From the middle of the floor of the fourth ventricle a small piece about the size of a pea is removed; this is triturated and thoroughly emulsified in a sterile watch-glass by means of a sterile glass rod with a bulbous end, a little sterile broth being used to make the emulsion, and sufficient being added to measure about 10 c.c. The hair on the head of a good-sized rabbit is cut close, the animal is anesthetized with ether, the skin on the scalp reflected, and a trephine hole made through the skull. The centre of the trephine hole should be in the middle line, and on the line drawn between the posterior corners of the eyes; the diameter of the trephine being about $\frac{1}{16}$ inch. A little of the emulsion is drawn up in a small syringe, having a fine needle, and two or three drops are injected beneath the dura mater. The operation is carried out with antiseptic precautions, the wound closed, and a little wool and collodion dressing applied.

If the material injected be from a rabid animal, the first symptoms will be noticed in from ten to fourteen days. The inoculated animal loses control over its hind legs and throws them about peculiarly when running. This increases, and in another day or so the animal is apt to fall when running, and in another day or two the hinder extremities become paralytic, and the animal is unable to move, and dies shortly. The onset of symptoms is hardly ever delayed beyond twenty-one days.

Van Gehuchten's Method.—The ganglion is placed in absolute alcohol for twelve hours, the alcohol being changed once; it is then embedded, and sections are cut. These are stained for five minutes in Nissl's methylene blue and mounted. Or the material may be fixed in 10 per cent. formalin before staining. The capsular changes are best shown by staining with hematoxylin and eosin.

Babes's Method.—A piece of the medulla or cord is hardened in alcohol and stained with anilin red, and sections are prepared.

MANUAL OF BACTERIOLOGY

MALIGNANT DISEASE.

analogies between carcinoma and sarcoma and other malignant diseases have lead investigators to search for organisms in these conditions.

Years ago Scheuerlein isolated a bacillus in carcinoma, but it was afterwards shown that the organism was one of the varieties of the *B. mesentericus*.

He was unable to isolate any bacterial form from other malignant disease. Doyen has isolated a micrococcus (*ibid.*, p. 208), but his results are not accepted.

Impetus was given to the study of parasites in malignant disease by the publication of a paper by Russell.²

By the use of certain methods of staining, small corpi were observed within the epithelial cells. They were spherical in shape, 10 μ in diameter, occurring singly or in groups, apparently homogeneous, and surrounded by a capsule. Russell regarded these structures as belonging to the fungi (blastomycetes) and they have since

of the cells of reproductive tissue (fig. 69, *b*). Save for the presence of these structures, there is no proof that protozoa are present in, or are the cause of, carcinoma.

Another hypothesis of the nature of malignant disease is that it is due to a blastomycotic infection. Sanfelice and others have isolated yeast-like forms which on inoculation apparently produce malignant growths. Critical examination, however, shows that these growths are granulomata and not true neoplasms. The same may be said of Washbourn's so-called infective venereal sarcomata of dogs.

Malignant disease occurs in all classes of vertebrates, and is generally inoculable on an animal of the same

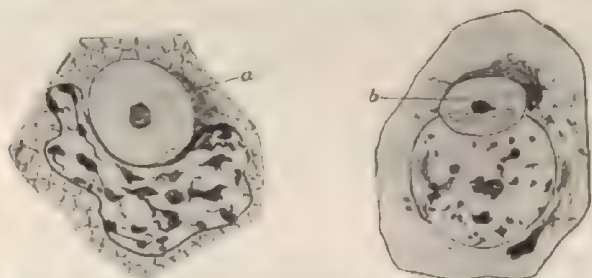


FIG. 69. *a*, REEFER'S OR PLIMMER'S BODY IN A CANCER CELL;
b, THE ARCHOPLASTIC VESICLE IN SPERMATID OF MOUSE. (After
FARMER, MOORE, and WALKER.)

species as that from which it is derived, but not on other animals. The carcinoma of mice has been the subject of much investigation of late. In the writer's opinion the trend of recent research is to show that malignant disease is not due to a micro-parasite, but is derived from the irresponsible division of cells of the normal or of embryonic tissues.¹

The mollusum bodies have likewise been regarded as parasitic (coccidial) in nature, but with them also inoculation and cultivation experiments have failed.

¹ For further information consult *Pathology, General and Special*, Ed. 2, R. T. Hewlett (Churchill, 1907).

MANUAL OF BACTERIOLOGY

EXAMINATION.

Russell's Bodies.—Treat sections as follows :

Wash in water.

Soak for ten to thirty minutes in a saturated solution of 2 per cent. aqueous carbolic.

Wash in water for a few minutes.

Put in absolute alcohol, half a minute.

Put in 2 per cent. solution of iodine green in 2 per cent. aqueous solution for five minutes.

Dehydrate in absolute alcohol, clear in oil of cloves, and mount in balsam.

Ruffer-Flemmer Body.—In order to demonstrate this rapidly growing visceral carcinoma should preferably be cut in thin slices. Pieces from the growing edge are fixed in corrosive sublimate (see below) or Flemming's solution. *Paraffin* sections are stained with haematoxylin, using as a contrast eosin or fast blue, or with the Ehrlich-Biondi reagent, or with anilin

oil. Ruffer recommends the following procedures :

Examination of Fresh Specimens.—Scrapings are made

this for twenty-four hours, then placed in 70 per cent. alcohol tinged with iodine (not Tinct. Iodi nor Gram's solution), then in increasing strengths of alcohol. Staining may be carried out with the Ehrlich-Biondi mixture (not usually satisfactory), with a concentrated aqueous solution of thionine blue fifteen to twenty minutes, dehydrating with acetone-alcohol (1 : 5), and passing through xylol; or with Mayer's hæmalum or Heidenhain's iron hæmatoxylin and counter-staining with orange-rubin.

Sanfelice recommends fixing in any ordinary fixing agent to which 2-3 per cent. of acetic acid is added, washing well, and hardening in increasing strengths of alcohol. The sections are stained with Ehrlich-Biondi and then with carbol-fuchsin diluted with three times its volume of water. After staining the preparation is treated with a mordant such as oxalic acid or diluted Lugol's iodine solution. The subsequent dehydration with alcohol does not then remove the stain from the parasite, which retains a deep violet colour.

VARIOLA AND VACCINIA.

The specific contagia of these two diseases have not yet been discovered with certainty.

Variola is inoculable on man, the calf and the monkey, vaccinia on the rabbit in addition.

A large number of observations have been made with vaccine lymph, but no distinctive bacterium has been obtained except by Klein and Copeman. Usually the ordinary pyogenic organisms and many saprophytic forms can alone be isolated. Klein observed the presence of a bacillus in vaccinia, which was subsequently more fully studied by Copeman.¹ It was found in vaccine vesicles at an early stage, but at maturation could no longer be detected. It is a very fine bacillus, and these observers were unable to cultivate it. Subsequently Copeman found a similar organism in variola, and succeeded in cultivating the bacillus from both sources in eggs, and from such egg-

¹ *Milroy Lectures on Vaccination*, 1898.

MANUAL OF BACTERIOLOGY

was able to inoculate calves. Klein¹ by storing crusts in 50 per cent. glycerin, and so getting rid of saprophytic forms, has cultivated an organism which is the *Bacillus albus variolæ*. Morphologically it resembles the bacillus observed in vaccine lymph; small, white, opaque, coherent colonies on agar, does not grow very feebly on gelatin. Involution forms occur, which seem to belong to the group of diphtheria and xerosis. On inoculation into calves some approach to, but not identical with, vaccinia was produced. Moreover, the inoculated calves were not immune to subsequent vaccination. Klein,² by inoculating collodion capsules filled with vaccine lymph, in which the bacilli had died out, and inserting in the abdominal cavity of rabbits, observed zooglyca masses made up of colonies resembling spores which he regards as the final stage of the specific microbe.

Korte finds that the vesicles, both in variola and in vaccinia, are sterile before maturation, and regards the

and Ruffer and Plimmer in this country described somewhat similar appearances. Ruffer and Plimmer¹ describe the supposed protozoon as a small round body, about $8\ \mu$ in diameter, lying within a clear vacuole in the protoplasm of the epithelial cell.

Councilman, Magarh, Brinkerhoff, Tyzzer, and Calkins² in America have found the Guarnieri body in variola and vaccinia in man and animals, and regard it as a protozoon and the causal agent of these diseases.

Ogata found bodies which he regards as parasitic protozoa and the causative agent of the disease in variolous and vaccine lymph. Reed likewise observed small granular amœboid bodies having a diameter of about one-third that of a red blood-corpuscle, similar apparently to those described by L. Pfeiffer, in the blood of vaccinated children and monkeys, but also observed them—and this is important—occasionally in the blood of normal children and monkeys.

Funck³ described a protozoan parasite as occurring under three forms: (1) round refractile green bodies $2-10\ \mu$ in diameter exhibiting movements upon a warm stage; (2) ovoid cells with lateral nuclei (epidermic cells) containing small brilliant green spheres $1-3\ \mu$ in diameter; (3) morula bodies $25\ \mu$ in diameter: these are cysts full of spores. Similar bodies are seen in variolous pustules. Roger and Weil⁴ describes somewhat similar structures in the contents of the variolous pustule. Calmette⁵ has also observed small refractile granules in lymph and has failed to cultivate any organism from it. The monkey and rabbit are both susceptible to vaccinia; in the latter animal the

¹ *Brit. Med. Journ.* 1894, i. p. 1412.

² *Journ. Med. Research*, xi., 1904, p. 173; *Philippine Journ. of Science*, i., 1906, p. 239.

³ *Brit. Med. Journ.* 1901, p. 448.

⁴ *La Presse Médicale*, Nov. 28, 1900.

⁵ *Ann. de l'Inst. Pasteur*, xv., 1901, No. 3, p. 161.

pustules are mature on the third day and immunity is acquired by the sixth day.

Ferroni and Massari state that appearances similar to those described by Guarnieri can be obtained in corneae inflamed by croton oil or Indian ink, and therefore believe that the so-called parasites are derived from the nuclei or from emigrated leucocytes. Salmon considers that the so-called parasites in vaccinia and variola are more or less condensed balls of chromatin of extra-epithelial origin derived from the migratory polynuclear leucocytes. According to Von Prowazch these cell inclusions (the Guarnieri bodies &c.) in this and other conditions (e.g. scarlatina) are not parasites, but consist of plastin and nuclease and are derived from the cells in which they occur.

De Korté¹ has observed in the variolous and vaccine vesicles before maturation large amœboid bodies (10 μ), which he believes to be protozoa (*Sporidium vaccinale*). In vaccine lymph refractile motile granules occur in abundance, believed by De Korté to be spores.

The relationship of vaccinia to variola has been a very vexed question. With few exceptions (Ceely, Hime, Simpson, Klein, King, Copeman), attempts to inoculate variola on the calf have failed. In the successful cases, the lymph obtained from the calf has, on inoculation upon children, produced typical vaccinia without any untoward results. The positive results obtained by the inoculation of variolous material being so few, a doubt arises whether in these cases there may not have been some fallacy, such as accidental contamination with vaccinia. Simpson, however, performed his experiments within the precincts of a smallpox hospital and away from possible vaccine infection, and Copeman² found that variola may be readily inoculated upon monkeys, and after several passages through these

¹ *Trans. Path. Soc. Lond.* lvi., 1905, p. 172.

² *Brit. Med. Journ.* 1901, i. p. 1134, and 1901, ii. p. 1736.

animals is easily inoculable upon the calf. He suggests, therefore, that vaccinia in the calf was originally due to infection with *inoculated* smallpox, so prevalent at the time of Jenner's discovery. A somewhat parallel instance of the attenuation of a virus by passage through another animal is recorded by Sticker and Marx in the case of bird-pox, which produces an extensive smallpox-like eruption in fowls and pigeons. In fowls and in pigeons the virus retains its pathogenic properties for each bird unaltered for any number of inoculations, but the pigeon strain, after a few inoculations into fowls, completely loses its virulence for the latter. There seems little doubt, therefore, that vaccinia is modified variola, and the rationale of vaccination rests upon a scientific basis.

The preparation of vaccine lymph is fully described by Blaxall.¹ Calves are vaccinated with lymph under aseptic precautions, and five days later the contents of the vesicles are scraped off, the pulp is triturated in a machine and is then placed in six times its weight of sterilized 50 per cent. pure glycerin in distilled water, and stored for about a month in test-tubes, until agar cultivations show that extraneous bacteria have died out, when it is issued for use. It remains very active for fifty to sixty days, after which it begins to deteriorate.

Green² rapidly prepares vaccine lymph by killing off the extraneous organisms with chloroform vapour.

¹ *Rep. Med. Off. Loc. Gov. Board* for 1898-99, p. 35.

² *Ibid.* for 1900-01, p. 639, and *Proc. Roy. Soc. Lond.* 1903.

CHAPTER XX.

DISEASES AND THEIR CAUSATIVE AGENTS—SOME DISEASES NOT PREVIOUSLY REFERRED TO—MICRO-ORGANISMS OF THE SKIN AND MUCOUS MEMBRANES.

APPENDICITIS.—The following table¹ shows the usual kinds and relative frequency of the infections in appendicitis :

Micro-organism	Acute Appendicitis	Chronic Appendicitis
<i>Bacillus coli</i> in pure culture	70 per cent.	90 per cent.
" with staphylococci	15 "	6 "
" streptococci	7 "	Very rare.
Staphylococci alone	4 "	1 per cent.
Streptococci "	Very rare.	Very rare.
Other organisms or combinations . . .	4 per cent.	3 per cent.

It is not improbable that in a still greater percentage of cases a mixture of organisms is present at first, the *Bacillus coli* subsequently crowding out the other forms. The *Bacillus proteus*, *B. pyocyaneus*, and *B. Welchii* also occasionally occur.

Castellani² describes a bacillus, pathogenic to guinea-pigs, isolated from a case of gangrenous appendicitis. Morphologically it resembled the Shiga-Kruse dysentery bacillus and was non-motile, produced acid and gas in glucose and maltose and curdled milk, but did not ferment mannite, lactose, and sucrose.

BERI-BERI.—Various observers have attempted to cultivate a micro-organism in this disease. Pekelharing and Winkler isolated a coccus producing a white growth and resembling

¹ Battle and Corner, *Diseases of the Vermiform Appendix*, 1904.

² *Brit. Med. Journ.* 1907, ii. p. 1513.

the *M. pyogenes*, var. *albus*, very closely. Hunter¹ obtained a similar coccus from two cases. He describes it as having a 'very active motion,' staining by Gram's method, producing white growths, and the earlier cultures liquefying gelatin slowly. It produced an acid reaction and formed indole. Some rabbits inoculated with it suffered from paresis, and the nerves were found to be degenerated.

A Gram-positive coccus was isolated by Okata and Kokubo from the blood and urine. It gives a greyish-white, granular moist growth on agar, a nearly clear fluid with sediment in broth, a shining greyish-white moist luxuriant growth on serum, on gelatin a somewhat delicate whitish growth, afterwards becoming yellowish without liquefaction, and on potato a light yellow, dry growth. Milk is not curdled.

Rost² described a small motile sporing bacillus which he isolated from the blood and cerebro-spinal fluid in cases of beri-beri. The organism is also present in rice, and can be cultivated in rice-water, ascitic fluid, or on blood serum. Inoculated into fowls it produced paresis and death.

Hamilton Wright suggests that the disease is due to an intoxication the result of a gastro-duodenal infection with a large Gram-positive bacillus (unisolated). Daniels believes that the epidemiology of the disease is best explained on the hypothesis of a protozoan infection conveyed by lice. The writer and De Korté³ also suggest a protozoan infection, the organism perhaps being eliminated in the urine.

Other views are that beri-beri may be a peripheral neuritis due to arsenical poisoning, or that it is an intoxication produced by bad rice, and the evidence in favour of the latter view seems to be accumulating.

BRONCHITIS.—Ritchie⁴ concludes that acute bronchitis is an infective disease, but is not due to any one specific organism, the most important causal bacteria being the *D. pneumoniae* and streptococci. In every case of acute bronchitis numerous

¹ *Lancet*, 1897, ii. p. 240 (Bibliog.).

² *Ind. Med. Gazette*, Dec. 1900, p. 458, and July 1901, p. 255.

³ *Journ. Trop. Med.*, Oct. 1, 1907, p. 315.

⁴ *Journ. Path. and Bact.* vii. No. 1, p. 1.

MANUAL OF BACTERIOLOGY

ic bacteria are present in the bronchi, which are usually healthy.

CRS, SOFT.—An extremely small bacillus, first described by,¹ has been found in the ulcers and buboes. It has not been cultivated successfully on animals, but can be inoculated into man, experimentally, from man to man. The bacillus is not stained by Gram's method, and can be cultivated on serum-agar, on which it forms shining greyish colonies 1 mm. in diameter, or in guinea-pig blood.²

CONJUNCTIVITIS.—Conjunctivitis is of several varieties :

Acute Contagious Conjunctivitis, due to the Koch-Weeks bacillus. This is a slender, non-motile organism, 1-1.5 μ in length, occurring singly or in pairs, both free and within the pus cells. It is not stained by Gram's method and is difficult to cultivate, but grows best on a serum-agar mixture, on which it forms small, transparent colonies. It is hardly pathogenic to man, but in man sets up a typical acute conjunctivitis.

Chronic Catarrhal Conjunctivitis, due to the Morax-Diemandt diplo-bacillus. This organism is 2 μ long by 1 μ broad, and is stained by Gram's method, and can be cultivated on

DIARRHŒA (SUMMER) OF INFANTS.—Booker¹ in an elaborate paper came to the following conclusions: 'No single micro-organism is found to be the specific exciter of the summer diarrhœa of infants, but the affection is generally to be attributed to the activity of a number of varieties of bacteria, some of which belong to well-known species, and are of ordinary occurrence and wide distribution, the most important being a streptococcus and the *Proteus vulgaris*.'

Lesage obtained a bacillus from the 'green diarrhœa' of infants which he believed to be the cause of this complaint. It is a small, motile, non-liquefying bacillus, producing on gelatin a whitish expanded growth with crenated margins, and giving rise to a green fluorescence in the medium.

In cases with blood and mucus in the stools, the *B. dysenteriae* (Shiga-Kruse type) has been found to be present in America and in this country. In London, Morgan has isolated in a number of cases a bacillus which in its fermentation reactions is nearly allied to the hog-cholera bacillus (see p. 851).

The stinking motions of the diarrhœa of children have been ascribed to the action of organisms belonging to the *Proteus* group, particularly *B. proteus* (*P. vulgaris*, see p. 597), which occurs in putrefying matter, sewage, and in the intestine. (This organism may also cause abscesses and cystitis, and a form of meat poisoning has been ascribed to its action.) Filtrates of cultures were found by S. Martin to produce fall of temperature, collapse, and diarrhœa in rabbits.

DISTEMPER OF DOGS.—According to Galli-Valerio,² this is caused by a bacillus (*B. canicula*) intermediate in character between the coli-typhoid and hæmorrhagic septicæmia groups of organisms.

DYSENTERY.—It must now be admitted that dysentery is a term applied to a series of clinical symptoms associated with colitis which is due to different specific agents. There are at least two forms of the disease, one, the so-called tropical or endemic dysentery, met with especially in the East, and characterized by chronicity, a tendency to relapses, amenability

¹ Johns Hopkins Hosp. Reps. vi., 1897, p. 159 (Bibliog.).

² Centr. f. Bakt. (Ref.), xli., 1908, p. 563.

MANUAL OF BACTERIOLOGY

with ipecacuanha, and the occurrence of the single case as a sequela; the other, epidemic dysentery, met all parts of the world, particularly in times of war and not amenable to ipecacuanha, and not followed by liver abscess. There are also probably other forms occurring in small or sporadically. With regard to the first or tropical dysentery, this is generally regarded as being due to the *Amœba dysenteriae*, which is found abundantly in the stools, especially in the acute form, and also in the liver abscesses (see p. 461).

The epidemic dysentery of Japan and other parts of the East is due to a single bacillus, or group of bacilli, has been isolated by Shiga, Strong, Kruse, and others. This is the *B. dysenteriae* (see p. 361).

Several observers have isolated from cases of dysentery a bacillus. Calmette in Tonkin isolated the *B. pyocyanea*, and this organism seems to have been the cause of a dysentery outbreak in New York State investigated by Lartigau.¹ In Japan, Ogata isolated a fine Gram-staining, liquefying bacillus which does not seem to have been met with by subsequent

cultures of these organisms, with or freed from their toxins, seem, however, to produce an impetigo rather than eczema. But the filtered cultures, i.e. toxins, are harmful to the skin, and when applied to it for one or two days by means of moist warm pads a typical papular or vesicular eczema ensues. Probably in the human subject in addition to the micro-organisms some peculiarity in the soil is necessary for the disease to develop.¹ In so-called seborrhœic eczema, a non-liquefying micrococcus which forms butyric acid has been isolated.

Impetigo.—The large vesiculo-bullous eruption of impetigo contagiosa is caused by the *Streptococcus pyogenes*; the small pustule in the neighbourhood of hair follicles, impetigo of Boeckhart, is caused by the *M. pyogenes*, var. *aureus*. The *B. diphtherie* may also cause an impetigo (p. 250).

Pemphigus.—A diplococcus has been isolated in acute pemphigus by Demme, and in the chronic form by Dähnhardt. Bulloch² and Russell Wells,³ in this country, seem to have isolated an identical organism, and the following description of it is taken from their papers. Cocci 0·8 to 1·5 μ in diameter, mostly arranged as diplococci, and staining by Gram's method. On surface agar the organism forms a thick, white, shining growth. In stab agar the growth has a 'nail-shaped' appearance. The colonies on agar are at first round, but later, in seven days, they throw out lateral projections and assume a rosette appearance. On gelatin the growth is slow and slight, with some, but not marked, liquefaction. On blood serum the growth resembles that on agar. On potato a whitish, semi-transparent film forms. Milk is curdled. In broth it causes a general turbidity, with a whitish sediment, and sometimes a pellicle, which soon sinks. Guinea-pigs and mice inoculated or vaccinated with the organism died in four to eight days, fine hæmorrhages occurring in the lungs, and the cocci being obtained from the blood. No bullæ appeared on the skin. The *B. pyocyaneus* may cause bullous eruptions (see p. 216).

¹ Galloway, *Practitioner*, Dec. 1901, p. 701; Whitfield, *ibid.* Feb. 1904, p. 202.

² *Brit. Jour. Dermatol.* viii., 1895, Nos. 91, 92, p. 159 (Bibliog.).

³ *Lancet*, 1896, i. p. 1219 (Bibliog.).

MANUAL OF BACTERIOLOGY

pyogenic cocci or their toxins may produce various eruptions, e.g. pemphigus neonatorum and contagiosus a gestationis.¹

Zoster.—Pfeiffer observed bodies in the cells of the which he believed to be protozoa. Gilchrist,² however, these merely as altered nuclei.

AND MOUTH DISEASE.—Streptococci and amoebæ have been isolated from the vesicles in this disease, but the former only only the *S. pyogenes*. Bussenius and Siegel³ isolated it, but a German commission comprising Löffler and Löffler and Frosch have determined that the organism is a very minute one, as it passes through the smallest celain filter.

FEVER.⁴—*Synonyms*: Rock, Mediterranean, or Undu-

A disease met with especially on the Mediterranean at also in South Africa, India, China, the Philippines, subtropical countries of America, and clinically often typhoid fever.

minute micrococcus (*M. mediterranea*), first described by

slightly orange colour, and round, with granular margins. On gelatin a whitish growth slowly forms without liquefaction, and in broth a diffuse cloudiness forms, with a white deposit, and without film formation. Litmus milk becomes alkaline without curdling. Alkali is also produced in glucose media, but galactose, maltose, and saccharose are unchanged (see table, p. 225). The distribution of the *M. melitensis* in the body corresponds closely with that of the *B. typhosus*: thus it is abundant in the spleen, relatively scanty in the blood, and is excreted in the urine.

The *M. melitensis* maintains its vitality outside the body in the dry state in dust or on clothing for two to three months, in tap or sea water for a month. The thermal death-point is about 55° C.

Inoculated into animals no result usually ensues; in the monkey, however, a febrile condition is produced, with enlarged spleen, sometimes terminating in death, the course of the temperature resembling that of the disease in man. By intracerebral inoculation Durham found that the organism becomes pathogenic for the rabbit and guinea-pig, otherwise it is without effect. For the diagnosis of the disease the agglutination reaction is most valuable. It may be carried out by the microscopic method, a 48-hours' broth culture being employed, the details of the process being the same as described at p. 172. Dilutions of 1 in 30, 1 in 50, and 1 in 100 should be prepared, as well as controls with normal serum, for old laboratory strains sometimes agglutinate with normal serum in dilution of 1 in 20 or 30 (see p. 170. Neglect of this precaution led Bentley to describe kala-azar as a Malta fever infection). The organism being minute, it is necessary to use the $\frac{1}{2}$ -inch oil-immersion, the $\frac{1}{4}$ -inch with a high eyepiece and draw-tube extended, or better a $\frac{1}{4}$ -inch dry objective. The sedimentation method is preferable.

The disease may be conveyed to monkeys by contact, by inhalation of infected dust, and by feeding. Mosquitoes and other insects do not seem to convey it.

The investigations of the Mediterranean Fever Commission¹ have shown that the main source of infection of man is by goat's

¹ Reports I.-VII., Royal Society, London, 1905-1907.

milk. Goats may be infected (and are largely so in endemic districts, e.g. Malta and South Africa) without showing any symptoms, and excrete the organism in large numbers in their milk. Since the goat's milk has been boiled the incidence of the disease in Malta has fallen from 668 cases in 1905 to 7 cases in 1907 in the Army, and in the Navy there were no cases in 1907.¹

Toxin, Vaccine, and Serum.—The *M. melitensis* forms no extra-cellular toxin, but Macfadyen obtained an endotoxin by disintegration. Attempts to prepare an anti-serum have not been successful. A vaccine prepared with cultures killed by heat (see p. 199) has been used in the chronic form of the disease by Bassett-Smith² and others with some amount of success.

MASTOID DISEASE.—See Otitis media.

MEASLES.—Doehle and Behla described small flagellated bodies which they believed to be protozoa in this disease. Canon and Pielicke found small bacilli in the blood, which Tchaikovsky confirmed. They are motile, do not stain by Gram's method, and can be cultivated on agar and serum, on which they form delicate colonies. Czajkowski has found a similar organism. Lesage³ cultivated a small micrococcus from the nasal mucus and blood, which produced a fatal hæmorrhagic septicæmia in animals. The influenza bacillus is found in many cases.

MENINGITIS may be caused by *D. pneumonia* (60 per cent. of acute cases), *D. intracellularis*, Still's diplococcus, *B. tuberculosis*, gonococcus and micrococci and streptococci.

MUMPS (EPIDEMIC PAROTITIS).—Mecray and Walsh⁴ have isolated from the parotid and blood in some cases of mumps a coccus resembling that described by Laveran and Catrin. It occurs chiefly as a diplococcus, but also in large groups. The colonies form circular, white, shining points, with slow growth and gradual liquefaction. On potato a white growth occurs; on blood serum a plentiful cream-coloured growth; and in litmus-milk, production of acid with coagulation.

NOMA AND CANCRUM ORIS.—Grawitz in 1890 observed bacilli in the affected tissues in this disease, others fusiform

¹ Bruce, *Nature*, May 14, 1908, p. 39.

² *Journ. of Hygiene*, vii., 1907, p. 115.

³ *Compt. Rend. Soc. Biol.* 1900, p. 203.

⁴ *Med. Record* (N.Y.), 1., 1896, p. 440 (Bibliog.).

bacilli with or without other organisms; Comba considered that there was probably no specific organism; Durante found the *M. pyogenes*, var. *aureus*, with *B. proteus*, and Ravenna the same micrococcus with the typhoid bacillus. Diphtheroid bacilli have also been isolated. Weaver and Tunnick¹ in a case of cancrum oris observed the presence of fusiform bacilli and spirilla. Hellesen² isolated a diplococcus from a case of noma. The organism is not unlike the pneumococcus, but possesses no capsule, is Gram-positive, gives a general turbidity in broth with acidity, forms no gas from glucose, curdles milk with acid production, and forms punctate, whitish-grey, translucent colonies on surface agar. On inoculation into animals a specific necrosis was produced.

Bishop and Ryan³ in two out of three cases isolated an organism which culturally and morphologically resembled the diphtheria bacillus, but which only produced some local inflammation on inoculation into guinea-pigs. In the third case the *M. pyogenes*, var. *aureus*, and the *Streptococcus pyogenes* were isolated. Guizzetti, and Freymuth and Petruschky⁴ have isolated the Klebs-Löffler bacillus in noma.

OPFLER-BOAS BACILLUS.—Met with in the stomach, particularly in cases of carcinoma, and its presence is suggestive of this condition. The bacilli occur in masses, are long and filiform and non-motile, and frequently join one another in angles. They measure usually 6-8 μ in length, but vary between 8 and 10 μ . The organism has been cultivated, and is a facultative anaerobe, non-sporing and Gram-positive. It curdles milk and forms lactic acid from various sugars.

OTITIS MEDIA.—The *Diplococcus pneumoniae* is perhaps the commonest organism met with; next in frequency comes the *Streptococcus pyogenes*, and then the pyogenic cocci. In scarlatinal otitis media, Blaxall⁵ found the *S. pyogenes* to be always present, and generally accompanied by other organisms, pyogenic cocci, &c. In thirty-seven cases of mastoid disease Blake⁶ found

¹ *Journ. Infectious Diseases*, iv., 1907, p. 8 (Bibliog.).

² See *Lancet*, 1908, i. p. 955.

³ *Journ. Amer. Med. Assoc.* xxv., 1895, p. 1043 (Bibliog.).

⁴ *Deutsch. Med. Woch.* 1898, pp. 332 and 600.

⁵ *Brit. Med. Journ.* 1894, ii. p. 113 (Bibliog.). * *Ibid.* 1897, i.

the following organisms, and remarks that as a rule the same were found in the middle ear :

Streptococcus	12
Staphylococcus	5
Diplococcus (? <i>pneumoniae</i>)	6
Streptococcus and diplococcus	5
Streptococcus and <i>Bacillus fetidus</i> (? colon bacillus)	3
Streptococcus and <i>Bacillus pyocyaneus</i>	1
Streptococcus and diplococcus	1
Streptococcus, micrococcus, and diplococcus	2

In two of the cases no organisms could be isolated.

OZÆNA (ATROPHIC RHINITIS).—Löwenberg described in this disease encapsuled bacilli somewhat resembling the pneumo-bacillus morphologically. Some Italian observers found bacilli apparently identical with the diphtheria bacillus. Abel¹ also described a bacillus somewhat resembling the pneumo-bacillus. It is this organism which produces the atrophy of the mucous membrane, but the fetor is due to the decomposition of the secretions produced by other organisms.

Perez² isolated an organism in ozæna (*Cocco-bacillus fetidus ozenæ*) which has the following characters : it is a short bacillus with rounded ends, non-motile, does not stain by Gram's method, does not liquefy gelatin, does not ferment lactose nor curdle milk, but forms indole and ferments urea. Its cultures are foul-smelling, and it is pathogenic for guinea-pigs, mice, rabbits, and pigeons.

PERITONITIS.—Treves³ gives the following table of the micro-organisms found in peritonitis :

	Fränkel	Tavel and Tausz	
	Found alone	Found alone	Found in association
<i>Bacillus coli communis</i>	11	15	16
Streptococcus	7	3	15
Staphylococcus	1	2	6
Pneumococcus	1	0	2
	20	20	39

¹ *Zeitschr. f. Hyg.* xxi. p. 89.

² *Ann. de l'Inst. Pasteur*, xiii. 1899, p. 937, and xv. 1901, p. 409.

³ *Brit. Med. Journ.* 1894, i. p. 229 *et seq.* (full Refs.).

Dudgeon¹ believes the *B. coli* is frequently a secondary agent and not the primary infection. He finds that the *M. pyogenes*, var. *albus*, is very commonly present from the first, and may exert a protective action by determining the occurrence of phagocytosis.

PERTUSSIS OR WHOOPING-COUGH.—Affanassieff observed large numbers of bacilli in the sputum in this disease. Koplik, by sowing the pellets on solidified hydrocele fluid, obtained a pure culture of a small and delicate bacillus measuring 0·8 to 1·7 μ in length. It formed a white growth, did not produce spores, and stained with methylene blue like the diphtheria bacillus, clubbed involution forms also occurring, and was pathogenic for mice, but not for guinea-pigs. It was isolated in thirteen out of sixteen cases.²

Czaplewski and Hensel³ isolated a bacillus having some similarities to, but differing in certain respects from, that of Koplik.

Davis⁴ states that in the sputum there is almost constantly present a bacillus morphologically and culturally identical with the influenza bacillus. Bordet and Gengou⁵ state that while this influenza-like bacillus is frequently present it is not the specific agent of the disease. This they believe to be a small bacillus exhibiting polar staining, difficult to cultivate except on a special medium (a 4 per cent. glycerin extract of potato with defibrinated rabbit or human blood solidified with agar), on which it forms a delicate growth.

Arnheim⁶ states that though the Czaplewski-Hensel and the Bordet-Gengou bacilli are very similar, they are not identical, and regards the latter as the causal agent, as it is agglutinated by the patient's serum.

Alberico⁷ describes a diplococcus.

PSILOSIS OR SPRUE.—Thin⁸ considers this disease to be due

¹ *Bacteriology of Peritonitis* (Constable, 1905).

² *Brit. Med. Journ.* 1897, ii. p. 1051 (Bibliog.).

³ *Centr. f. Bakt.* xxii. 1897, pp. 641 and 721, and xxiv. 1898, p. 865.

⁴ *Journ. Infect. Diseases*, iii., 1906, p. 1 (Bibliog.).

⁵ *Ann. de l'Inst. Pasteur*, xx. 1906, p. 731, and xxi. 1907, p. 720.

⁶ See *Brit. Med. Journ.* 1908, ii. p. 523.

⁷ *Ibid. Epit.* p. 32. ⁸ *Psilosis or Sprue* (J. & A. Churchill, 1897).

to an abnormal fermentation in the intestine brought about by some organism which has not yet been isolated.

PUERPERAL FEVER.—This condition may be either a localized infection with intoxication (sapremia), or a localized infection with general infection (puerperal septicæmia); in both the primary seat of infection may be perineal or vaginal lacerations, or the contents of the uterus or the placental site. The infecting organisms may be *S. pyogenes*, pure (20 per cent.) or with other organisms (80 per cent.), occasionally the *D. pneumoniae*, *B. coli*, *M. pyogenes*, var. *albus*, *M. pyogenes*, var. *aureus*, *M. gonorrhoea*, *B. Welchii*, and diphtheroid bacilli. These are rarely alone, but generally occur with one or other of the organisms named. The *B. diphtheria* may exceptionally be met with.¹

PURPURA.—Hæmorrhagic septicæmia may be caused by a number of capsulated bacilli allied to the *B. pneumoniae* of Friedländer,² as well as by streptococci and pyogenic cocci. Paratyphoid infection may be accompanied with purpura.

PYORRHOEA ALVEOLARIS (Rigg's Disease).—Goadby³ believes the *M. pyogenes*, var. *albus*, *S. salivarius*, *S. pyogenes*, and *M. pyogenes*, var. *aureus*, to be the causative organisms in various cases, and has used vaccine treatment with success. Whittles has described a Gram-staining bacillus.

RHEUMATISM (ACUTE).—The opinion has gained ground of late years that acute rheumatism is an infective disease. A number of observers have isolated streptococci and micrococci in this disease, and Singer⁴ regards the disease as merely an attenuated form of pyæmia. Menzer considers that rheumatic fever is not due to any one organism, but is a particular reaction in predisposed persons to various microbes, especially streptococci. In 1897 Achalmé⁵ isolated an anaërobic anthrax-like bacillus from several cases. This bacillus agrees in all its characters with the *B. Welchii* (*enteritidis sporogenes*), and is believed by the writer⁶ to be identical with the latter; it is probably a

¹ See Foulerton, *Practitioner*, March 1905, p. 387.

² See p. 235. Also Howard, *Journ. Exp. Med.* iv., 1899, p. 149 (Bibliog.).

³ *Brit. Med. Journ.* 1908, ii. p. 477; *Lancet*, March 9, 1907.

⁴ *Berl. klin. Wochr.* 1899, No. 33, and *Wien. klin. Woch.* 1901, No. 20.

⁵ *Ann. de l'Inst. Pasteur*, xi., 1897, p. 815.

⁶ *Trans. Path. Soc. Lond.* lii. Pt. ii. 1901, p. 115.

terminal infection or a contamination. Poynton and Paine¹ in 1899 obtained from eight successive cases a diplococcus (*D. rheumaticus*) possessing the following characters: minute cocci averaging $0.5\ \mu$ in diameter, often associated in pairs, but in liquid media forming chains. The organism grows both aerobically and anaerobically, but does not thrive well upon ordinary media, developing best in milk broth slightly acidified with lactic acid or upon blood agar—on the latter medium small raised yellowish-white discrete colonies develop in twenty-four hours. Injected intravenously into rabbits, the diplococcus frequently produces enlargement and inflammation of the joints with effusion, and occasionally valvulitis and endocarditis. In man the organism was demonstrated in the vegetations, pericardium, tonsils, and rheumatic nodules, and has been isolated from the blood, pericardial fluid, cardiac vegetations, and tonsils.

Andrewes and Horder found that two strains of the *D. rheumaticus* corresponded with the *S. faecalis* (p. 218).

RHEUMATOID ARTHRITIS (ARTHRITIS DEFORMANS). — Schuller described a small bacillus in this disease.² Blaxall³ found in the synovial fluid, and occasionally in the blood, a minute bacillus measuring $2\ \mu$ in length. It possessed marked polar staining, was decolorized by Gram's method, and could only be stained by prolonged immersion in the solution. Blaxall obtained the best results by fixing cover-glass specimens by passing six times through the flame, treating with dilute acetic acid for two minutes, washing and drying, then staining in anilin methylene blue for three to five days, washing in running water for some hours, rinsing in distilled water, drying and mounting. The organism can be cultivated on agar, on serum, and in broth. In a clear broth, after three days, minute shining, yellowish particles appear and increase in amount, giving rise on shaking the flask to an appearance of 'gold dust.' On agar and on serum an extremely delicate film of growth appears, only visible with a lens. In cultivation the bacillus is found to be non-motile, to form zoogloea masses, and sometimes to grow into longer

¹ *Lancet*, 1900, ii. p. 861 *et seq.*; *Trans. Path. Soc. Lond.* lii., 1901, Pt. iii. p. 248; *ibid.* lv., 1904, p. 126.

² *Berl. klin. Woch.* Sept. 4, 1893. ³ *Lancet*, 1896, i. p. 1120 (Bibliog.).

individuals. It does not grow on gelatin. Inoculation experiments on animals failed.

Poynton and Paine¹ have isolated a diplococcus (? a form of their *D. rheumaticus*) from an osteo-arthritic joint, which produced arthritis, with osteo-arthritic changes, when injected intravenously into rabbits.

Osteo-arthritis is probably not a single disease.

RHINOSCLEROMA.—A bacillus has been described in this disease. It is a short rod, with rounded ends, encapsuled, and frequently linked in pairs. The organism is non-motile, does not stain by Gram's method, and forms on gelatin a whitish growth without liquefaction like that of Friedländer's pneumo-bacillus. Milk is not coagulated. The organism is slightly pathogenic. It is doubtful if it is the causal agent.

RINDERPEST.—Simpson, Koch and Eddington described bacilli in this disease, but Nicolle and Adil-Bey have found that the virus passes through a porcelain filter, and the organism therefore is probably ultra-microscopic.

SYPHILIS.—A small bacillus was observed by Lustgarten² in the lesions in this disease, a large bacillus by Eve and Lingard, a strepto-bacillus by Van Niessen, and a Gram-negative polymorphous bacillus by Justin de Lisle and Jullien, but these are generally believed to be accidental organisms.

In February 1905 Siegel³ claimed to have discovered the parasite of syphilis, which he named the *Cytoryctes luis*. He described it as occurring as a motile, pear-shaped, refractile body 0.5–1 μ in length, subsequently breaking up into small refractile non-motile spores.

In March 1905 Schaudinn⁴ noted the constant presence of a spiriform organism or spirochaeta (*S. pallida* or *Treponema*

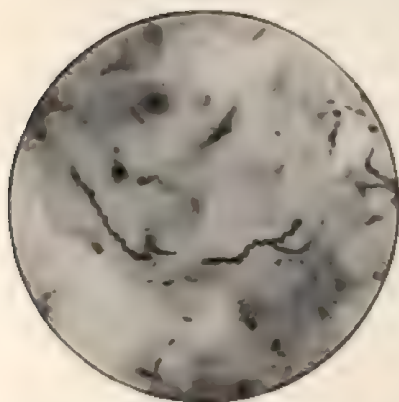
¹ *Brit. Med. Journ.* 1902, i, p. 79.

² Lustgarten stained his bacillus as follows: Sections are treated with anilin gentian-violet for twenty-four hours, washed in alcohol, decolorized in 1.5 per cent. potassium permanganate for ten seconds, rinsed in 5 per cent. sulphuric acid, washed in water, and mounted. If not sufficiently decolorized before mounting, the treatment with permanganate and sulphuric is repeated.

³ *Anh. Abhandl. Akad. Wiss. Berlin*, 1905.

⁴ *Arbeit. a. d. Kais. Gesundheitsamte*, xx., 1905.

PLATE XXIV.



a. *TREPONEMA PALLIDUM*.
SECTION OF LIVER OF FETUS (CONGENITAL SYPHILIS).
LEVADITI'S METHOD. × 1500.



b. *TREPONEMA PALLIDUM*.
SMEAR FROM CONDYLOMA. GIEMSA. × 1500.



pallidum) in various lesions in acquired and congenital syphilis. The *T. pallidum* varies from 4-10 μ in length, averaging 7 μ (Plate XXIV. *b*). It is much more attenuated than the majority of spirochaetes, having a maximum thickness of 0.5 μ , has three to twelve twists forming a close and narrow spiral, is actively motile, though possessing no flagella, but may have an undulating membrane. It stains feebly and with difficulty. Another spirochaete, the *S. refringens*, frequently accompanies, and must not be mistaken for, the *T. pallidum*; the former is more refractile and coarser, has fewer twists and forms a wider spiral, and stains deeper and more readily than the latter. The *T. pallidum* has been found in primary and secondary lesions of syphilis, but very rarely in the blood and tertiary lesions; it is abundant in the organs in the fetus in congenital syphilis¹ (Plate XXIV. *a*).

The *T. pallidum* has of late been assumed to be the specific organism of syphilis, being present not only in human lesions but in experimental lesions of inoculated apes (see below). The writer still hesitates to accept this as proved, because spirochaetes seem to be organisms of constant saprophytic occurrence, e.g. the *S. refringens* always accompanies the *T. pallidum* in chancres, spirochaetes are found in malignant tumours, ulcers, ulcerating granuloma, yaws, &c.

The *T. pallidum* has not been cultivated *in vitro* in spite of numerous attempts. By placing material from a rhesus monkey inoculated with syphilis into collodion sacs and introducing them into the peritoneal cavity of another monkey, and examining the contents of the sacs a month after the operation, a great multiplication of the organism was found to take place.²

Metchnikoff and Roux (also Grünbaum) found that the chimpanzee is very susceptible to syphilis, and can readily be inoculated from man, the *T. pallidum* being found in the lesions.

Macacus rhesus is also somewhat susceptible, likewise the *M. cynomolgus* and the Chinese bonnet monkey, but not the

¹ On the occurrence of the *T. pallidum*, see Schaudinn, *Arbeit. a. d. Kais. Gesundheitsamte*, xxii.; Buschke and Fischer, *Deut. med. Woch.* May 19 and 23, 1905; Levaditi, *C. R. Soc. Biol.* May 20 and June 17, 1905.

² Levaditi and McIntosh, *Ann. de l'Inst. Pasteur*, xxi., 1907.

MANUAL OF BACTERIOLOGY

By several passages through a *rhesus* monkey the virus becomes attenuated, so that in man it produces only a local lesion.¹ Bertarelli² states that syphilis may be produced on the rabbit.

Attempts by Metchnikoff and Roux to prepare an anti-syphilitic serum by inoculating apes and goats with syphilitic virus proved unsuccessful (as did earlier experiments with other animals by Héricourt and Richet). The syphilitic virus as introduced into man by sexual intercourse probably requires some hours to become generalized, for Metchnikoff found experimentally in apes that if the seat of inoculation were treated with a mercuric ointment up to eighteen hours after inoculation the disease was prevented.

Porté³ has described many curious bodies in the chancre, in the skin, and particularly in the blood during the secondary stage. The form which occurs most abundantly appears as a darkly staining, spherical body, measuring from 2 μ to 4 μ and occasionally a small central refractile nucleus is made out. This form when unstained resembles a red blood corpuscle, for which it may be mistaken. These bodies

a cubic centimetre of distilled water, and rendering alkaline with a few drops of 1 per cent. potassium carbonate solution.) The preparations may also be stained in the undiluted Giemsa stain for half to six hours.

So confident are most authors that the *T. pallidum* is the causative organism of syphilis, that its demonstration is held to be a means of diagnosing the disease.

2. *Levaditi's method* may be employed for sections:

(1) Fix pieces of tissue about 1 mm. thick in 10 per cent. formalin for twenty-four hours.

(2) Wash in water, and harden in 96 per cent. alcohol for twenty-four hours.

(3) Wash in distilled water for some minutes (until pieces sink).

(4) Place in 8 per cent. silver nitrate solution at 37° C. for three to five days in the dark.

(5) Wash in distilled water for some minutes, and then place in the following solution at room temperature for twenty-four to forty-eight hours:

Pyrogallie acid	2-4 grams
Formalin	5 c.c.
Distilled water	100 c.c.

(6) Wash in distilled water, dehydrate in absolute alcohol, clear in xylol, embed in paraffin, cut, and mount.

The spirochaetes are stained black or brown (Plate XXIV., a), the tissues yellow.

Some have asserted that the spirochaetes seen in the tissues after staining by this method are artifacts or are composed of filaments of elastic tissue.¹

Antigen Test.—This has been largely applied as a confirmatory test of the presumably syphilitic nature of such conditions as tabes dorsalis and general paralysis of the insane. In these conditions, if syphilitic, the infection having lasted a long time, the patient's blood should contain syphilitic anti-bodies, including the specific immune body. The test is based on complement fixation (p. 165). In this method a specific organism fixes its

¹ See Saling and Mühlens, *Centr. f. Bakt. (Orig.)*, xlii. and xliii.

homologous immune body, and the complex then takes up complement; this is demonstrated by the use of a hæmolytic system (p. 166). But in syphilis the organism cannot be cultivated, and therefore some other means had to be devised to provide the substance which fixes the immune body. For this purpose an extract of the organs (e.g. liver) of a syphilitic fetus is employed. The disease being at an early stage, an abundance of the specific organism and its products (the 'antigens,' p. 189) are presumably present and little anti-serum has been formed, and the antigens will fix the homologous immune body. The requirements are—(1) antigen extract; (2) syphilitic immune body; (3) complement; (4) a hæmolytic system; (5) the test fluid, i.e. the serum or cerebro-spinal fluid of the patient to be tested; (6) body fluid, corresponding to the test fluid, but derived from a normal, non-syphilitic person.

1. *The antigen* is prepared as follows: The liver of a syphilitic fetus is obtained as fresh as possible, and at once manipulated. It is finely minced and well triturated with the addition of 1 to 4 parts of 0·5 per cent. carbolic in physiological salt solution. The mixture is then shaken for twenty-four hours, centrifugalized, and the clear extract (the antigen) is kept in a refrigerator (Wassermann). Levaditi takes the fresh liver, minces it fine, and dries over H_2SO_4 *in vacuo*. One gram of this powder is rubbed up with 80 c.c. of physiological salt solution allowed to stand for twelve hours in a refrigerator, then centrifugalized, and the clear extract (the *antigen*) used. Landsteiner¹ makes use of an alcoholic extract of the liver. The antigen extract should be clear, and should have the following properties: (1) 0·2 c.c. extract + 0·1 c.c. known syphilitic blood serum (e.g. derived from a well-marked case of tertiary syphilis) + complement + hæmolytic system = no hæmolysis; (2) 0·2 c.c. extract + 0·1 c.c. normal serum + complement + hæmolytic system = complete hæmolysis; (3) 0·5 c.c. extract + complement + hæmolytic system = the hæmolysis should be as complete as in (2).

For purposes of control an extract of *normal* liver should be prepared in a manner similar to that employed for making the antigen extract.

¹ *Wien. klin. Woch.* Dec. 12, 1907.

2. *The syphilitic immune body* is represented by blood serum, or cerebro-spinal liquid, of a definitely syphilitic patient with tertiary symptoms. The serum or fluid is inactivated by heating for thirty minutes to 56° C.

3. *The complement* is guinea-pig serum in tenfold dilution. It keeps unaltered for a long time at -12° C.

4. *The Hæmolytic System*.—The hæmolytic serum is obtained from rabbits which have received repeated intravenous injections of washed sheep's blood-corpuscles (see p. 167). It is inactivated, and keeps well in the ice safe. It is used in double the concentration which suffices on trial for complete hæmolysis. The sheep's blood-corpuscles must be fresh. They are freed from serum by repeated washing with saline solution, and are employed in a 5 per cent. suspension. The hæmolytic system with complement should give prompt and complete hæmolysis.

5. *The test fluid* is the patient's serum or cerebro-spinal fluid inactivated by heating to 56° C. for thirty minutes.

6. *The body fluid* (corresponding to the test fluid) of a normal person is also inactivated by heating to 56° C. for thirty minutes.

The quantity of each of these six reagents employed is made up to 1 c.c. with saline solution. The volume of fluid in the controls is also made up to 5 c.c. with saline solution.

The following mixtures are then prepared :

1. *Controls* : (a) Saline solution + antigen extract ; (b) the test fluid + normal liver extract ; (c) the test fluid + saline solution ; (d) normal body fluid + antigen extract ; (e) syphilitic immune body + normal liver extract ; (f) syphilitic immune body + antigen extract.

2. *Test mixture* : the test fluid + antigen extract. To every mixture complement is added, and after the mixtures have stood for three to four hours at 20° C., the requisite amount of the hæmolytic system is also added, and the mixtures are allowed to stand for a further three to four hours, at the end of which time the occurrence or non-occurrence of hæmolysis is noted. If everything is right, hæmolysis should occur in all the controls except the last one, (f).

If the test fluid under examination is derived from a patient suffering from a syphilitic condition, it should contain syphilitic immune body, and hæmolysis will *not* take place.

The examination of a very large number of cases of syphilis by different observers indicates that the test is of considerable value and diagnostic significance. In conditions such as *tabes dorsalis* and general paralysis of the insane, which on other grounds are generally regarded as due to syphilis, 52 per cent. give the reaction. A *positive* reaction may be said to show a positive, and probably active, syphilitic infection, but a *negative* reaction does not necessarily exclude syphilis. A course of mercurial treatment may render the reaction negative.¹

Further research suggests that the active substance in the antigen extract is not a true antigen, for it is soluble in alcohol, but is a lipid, fatty, or other substance, or cholesterol; and Levaditi and Yamanouchi² have found that sodium taurocholate, glycocholate, or oleate, lecithin, and cholesterol, all act as an antigen. It has likewise been found that the reaction is not specific for syphilis, but occurs also in yaws and sleeping sickness. On these grounds, Müller³ suggests a simplified technique in which the antigen extract made from syphilitic liver is represented by an extract of heart muscle prepared as follows: One gram of fresh guinea-pig heart muscle is finely minced and ground up with powdered glass or quartz sand; this is then mixed with 50 c.c. of 95 per cent. alcohol, kept at 60° C. with occasional shaking for two hours, filtered through paper and preserved in a stoppered bottle at room temperature. This extract *acts as an antigen*. The following mixture is prepared: 10 drops of physiological salt solution, 1 drop of the inactivated serum to be tested, 1 drop of complement, and 2 drops of the alcoholic extract of muscle. The mixture is kept for one hour at 37° C., and then a drop of the hæmolytic system mixture is added.

TRACHOMA.—Sattler described a diplococcus in this disease. It is strictly aerobic, forms a scanty whitish growth on gelatin,

¹ See Citron, Wassermann, and Meier, *Journ. Roy. Inst. Public Health*, xv., 1907, pp. 560-563 (abstract); Lesser, *Brit. Med. Journ. Epit. i.*, 1908, p. 66; Marie and Levaditi, *Ann. de l'Inst. Pasteur*, Feb. 1907.

² *Compt. Rend. Soc. Biol.* xiv. pp. 169, 313, 349, 720.

³ *Wien. klin. Woch.* 1908, No. 9.

which is not liquefied, and a copious viscid white growth on agar and on serum.¹

Griffith regards trachoma as being caused by the Koch-Weeks bacillus.² The Morax-Axenfeld diplobacillus and the pneumococcus have also been isolated from cases. The causative organism cannot yet be said to be known.

TYPHUS FEVER.—Lewaschew³ states that he has observed in the blood of patients suffering from typhus fever round, highly refractile bodies, the poles of which are occasionally elongated so as to form a flagellum on either side. The organism can be cultivated by smearing droplets of the blood on agar-tubes and incubating at 37° C. In gelatin stab-cultures liquefaction occurs. The organism is strictly aerobic, and inoculation experiments failed. McWeeney⁴ obtained negative results by culture methods. Various cocci and spherical organisms have been described by several observers.

The disease is usually considered to be very infectious, but Matthew Hay,⁵ discussing the Aberdeen epidemic of 1905-6, notes that no nurse or wardmaid, however intimate her contact with the typhus cases, or however prolonged the exposure to infection, who did not assist in removing patients to hospital, or in cleaning them, was attacked—for example, all the nurses on night duty in the acute wards, and all the nurses, both day and night, in the convalescent wards. In the same category may be placed the doctors and the matron. Hay suggests, therefore, that fleas may be the agents which transmit infection.

VARICELLA.—Various cocci have been isolated. De Korté described an amœboid protozoan-like body.

YAWS.—Castellani⁶ has found in the yaws granulomata a delicate spirochaete resembling the *S. pallida* of syphilis closely, but even more delicate and difficult to stain than the latter organism, and named the *S. pertenuis*. It is present also in the

¹ See Lawson, Roy. Lond. Ophthalm. Hosp. Reps. xiv., 1897, p. 484.

² Thompson Yates Lab. Rep. iv. Pt. i. 1901, p. 139.

³ Arch. des Sc. Biolog. de l'Inst. Imper. de Méd. Expér. de St.-Petersbourg, iv., 1896, No. 4.

⁴ Brit. Med. Journ. 1898, i. p. 881 (Bibliog.)

⁵ Public Health, xix., 1907, p. 772.

⁶ Brit. Med. Journ. 1907, ii. p. 1511.

spleen and lymphatic glands in the disease and in inoculated monkeys.

Some observers have supposed yaws to be a manifestation of syphilis, but (1) syphilitic patients can be inoculated with yaws; (2) syphilis may supervene on yaws; (3) Neisser and Castellani have shown that monkeys inoculated with syphilis are not immune to yaws, and *vice versa*; and (4) Castellani¹ has shown that the yaws antigen and anti-bodies are distinct from the syphilis antigen and anti-bodies. Wise has found spirochaetes in the ulcerating granuloma of the pudenda of tropical countries.

YELLOW FEVER.—As far back as 1889 Sternberg described a bacillus—'*Bacillus X*'—in yellow fever, a facultative anaërobic organism, very pathogenic to rabbits. Then in 1897 Sanarelli² described his *Bacillus icteroides*, which later investigation has proved to be an organism belonging to the Gärtner group (see p. 351).

Considerable controversy arose with regard to the ætiological significance of this organism. Sternberg claimed that it is identical with his *Bacillus X*. An American commission³ investigated the matter and came to the conclusion that the *Bacillus icteroides* is the cause of yellow fever, that it is met with only in yellow fever, that it is agglutinated by the blood serum of yellow-fever patients but not by other sera, that it reproduces on inoculation the lesions (e.g. fatty degeneration) characteristic of the disease in man, and that it is a species distinct from the *Bacillus X*.

Later, another Board of American medical officers⁴ investigated yellow fever in Cuba; eighteen cases were examined during life with a view to the isolation of Sanarelli's bacillus, blood being taken from a vein and cultures made on various media, but with entirely negative results. Similarly, eleven cadavers were investigated, also with negative results.

Agramonte failed to obtain agglutination of the *B. icteroides*

¹ *Journ. of Hygiene*, vii., 1907, pp. 558.

² *Ann. de l'Inst. Pasteur*, xi., 1897, pp. 433, 673, and 753.

³ *Med. News* (N.Y.), Dec. 9, 1899, p. 737.

⁴ *Philad. Med. Journ.* Oct. 27, 1900, p. 790.

with yellow fever serum, and the latter did not protect animals against infection with the *B. icteroides*.

Reed and Carroll¹ critically examined the *B. icteroides* and its relation to yellow fever. Their conclusions were that the *Bacillus X* belongs to the colon group, the *B. icteroides* to the Gärtner group, that the *B. icteroides* and hog-cholera bacillus produce the same lesions in animals and mutually protect against each other, that the *B. icteroides* causes in swine all the symptoms and lesions of hog cholera, and that the blood of hog cholera agglutinates the *B. icteroides* in a much more marked degree than does the blood of yellow fever.

Reed, Carroll, and Agramonte² having thus shown the etiological position of the *B. icteroides* to be untenable, directed their attention to the transference of yellow fever through the agency of mosquitoes. Finlay, of Havana, suggested many years ago that yellow fever might be propagated through the intermediary of a mosquito—*Stegomyia calopus* (*fasciata*)—and with this species these investigators worked. They allowed mosquitoes to bite yellow-fever patients at various stages of the disease, and the infected mosquitoes were subsequently allowed to bite eleven individuals, two of whom contracted yellow fever. It is true this is not a very convincing experiment, but it is to be noted that during the period of fifty-seven days among a population of 1400 non-immune Americans there were only three cases of yellow fever, and that two of these had been bitten by contaminated mosquitoes within five days of the commencement of their attacks. The matter was put to the further test of experiment in the following manner.³ Under the same observers a camp was established with several tents each occupied by one to three non-immune individuals, and precautions were taken to prevent the introduction of yellow fever from outside. Five individuals were bitten by infected mosquitoes, and four out of the five contracted yellow fever, no other occupants of the camp being attacked by the disease. Subsequently several non-immune individuals were exposed to yellow-fever infection from soiled

¹ *Journ. Exper. Med.* v. Pt. iii, p. 215.

² *Philad. Med. Journ.* Oct. 27, 1900, p. 790.

³ *Journ. Amer. Med. Assoc.* Feb. 16, 1901, p. 431.

linen, yellow-fever discharges, &c., in a mosquito-proof hut from which mosquitoes were excluded, with entirely negative results. These experiments prove, therefore, that yellow fever is conveyed by mosquitoes only, and further work by Americans and Cubans, and by French and Brazilian Commissions, have entirely confirmed these researches and conclusions. It has been found that to convey infection the mosquitoes need to bite the patient during the first three or four days, but do not become infective until about the twelfth day after feeding, and retain their infectivity for five days or so. All these facts point to a protozoon as being the causative organism, but none has been found.

Durham and Myers¹ searched in vain for a protozoan form in yellow fever. In the tissues they found a small bacillus which stained only with great difficulty (by immersion for some hours in weak carbol-fuchsin), and which could not be cultivated by ordinary means. The Americans have shown that the blood serum after filtration through a porcelain filter is still infective; the organism, therefore, is probably ultra-microscopic.

MICRO-ORGANISMS OF THE SKIN AND MUCOUS MEMBRANES.

Skin.—A very large number of micro-organisms are always present on the skin of every part of the body, and it is impossible to touch the smallest point without obtaining a culture if an inoculation be made. The pyogenic cocci² are everywhere present, a variety of the *M. pyogenes*, var. *albus*, the *M. epidermidis* (*albus*) of Welch, being one of the commonest (see p. 207). Equally common on the skin and scalp is the scurf micrococcus isolated by Gordon (see table, p. 207). Sarcinæ, bacilli, and moulds are also present. On the skin of the groin, scrotum, and vulva the smegma bacillus occurs. From sweating feet various organisms have been isolated, which on culture evolve a disagreeable odour, among which is the *Bacterium fetidum* of Thin.

Conjunctive.—Some observers have stated that the conjunctiva is generally sterile. A certain number of organisms are, however, usually present, though they are not numerous, and if

¹ *Brit. Med. Journ.* 1901, i. p. 450.

² On the rôle of cocci in the pathology of the skin, see *Brit. Med. Journ.* 1901, ii. p. 794.

artificially inoculated the excess is rapidly eliminated. The *B. xerosis* can often be isolated.

Randolph¹ states that the normal conjunctiva always contains organisms, the commonest species being the *Micrococcus epidermidis* (*albus*) of Welch.

Lawson² found the normal conjunctiva to be sterile in 20 per cent. of cases and pyogenic cocci to be rare, and, when present, non-virulent.

Nose.—In the anterior nares, crusts and vibrissæ micro-organisms are present in great abundance, but, contrary to the usual opinion, St. Clair Thomson and the writer³ showed that the mucous membrane of the interior of the nose is comparatively sterile, and when organisms are present they are very scanty compared with the number of organisms inspired.⁴ Moreover, organisms artificially deposited were found to be rapidly disposed of. After two hours, for example, *prodigiosus* inoculated on to the inferior turbinate could not be detected by cultivation. Wurtz and Lermoyez asserted that the nasal mucus is germicidal, but St. Clair Thomson and the writer⁵ were unable to confirm this, though it may have an inhibitory action.

Air Passages.—Below the larynx under normal conditions the air passages are free from micro-organisms. Expired air is also free from organisms, and the air from the naso-pharynx after passing through the nasal cavities is deprived of the majority of its organisms.⁶

Mouth.—Micro-organisms of all kinds are present in the buccal cavity in the greatest abundance—leptothrix, bacilli, pyogenic cocci, sarcinae, and spirilla are almost always to be found. The *Streptococcus pyogenes*, *M. pyogenes*, var. *aureus*, and *Diplococcus pneumoniae* are frequently present. Certain organisms have their normal habitat in the mouth, are difficult to cultivate,

¹ Archives of Ophthalmol. xxvi., 1897, p. 379.

² Trans. Jenner Inst. Prev. Med. ii. p. 56; also Griffith, Thompson Yates Lab. Rep. iv. Pt. i. 1901, p. 99.

³ Medico-Chirurg. Trans. 78, 1895 (Bibliog.).

⁴ Other observers, however, have not altogether confirmed this. See Iglauer, Laryngoscope, 1901, Nov., p. 363.

⁵ 'The Fate of Micro-organisms in Inspired Air,' Lancet, 1896, Jan. 11.

⁶ Ibid.

MANUAL OF BACTERIOLOGY

of considerable importance in the production of dental caries. Well-defined micrococci and streptococci also occur in saliva (*M. salivarius*, p. 207, and *S. salivarius*, pp. 213 and 214). The normal saliva is germicidal to some extent. (See p. 35, 486).

Mouth and Intestine.—Although a vast number of organisms gain access to the stomach, a large number are destroyed by gastric juice. At the same time a considerable proportion do survive—sarcinae, and lactic and butyric acid bacilli. In nurslings the mouth and stomach contain few bacteria, cocci, and some bacilli of the *B. coli* and *B. lactis aerogenes* type. The small intestine contains remarkably few organisms of any type. In the large intestine bacteria are extremely numerous, particularly Gram-positive ones. These are mostly slightly curved, bacilli of moderate size, the *B. bifidus* type, which often has a bifid extremity, also a somewhat acid-fast organism, *B. acidophilus* of Moro, but capable of decolorization in an acid medium, a few *B. Welchii*, and a diplococcus. In non-negative forms are *B. coli*, *B. lactis aerogenes*, and in bottle-fed children the same organisms occur, but the

increase in number in inflammatory conditions, and Gram-negative cocci may be found (see p. 222). The deeper portion of the urethra, however, is free from organisms, and the bladder is sterile. The genital tract in the female up to the middle zone of the cervix contains organisms, but the uterus and Fallopian tubes are normally sterile. The *B. vaginæ* of Döderlein, a large Gram-positive bacillus capable of growing in an acid medium, is frequently present in considerable numbers in the vagina.

MANUAL OF BACTERIOLOGY

CHAPTER XXI.

BACTERIOLOGY OF WATER, AIR, AND SOIL, AND THEIR
BACTERIOLOGICAL EXAMINATION — SEWAGE — BACTERIOLOGY
MILK AND FOODS.

of the Commoner Organisms found in the Air, Water, and Soil.

THE BACTERIOLOGICAL EXAMINATION OF WATER.¹

The bacteriological examination of water supplies affords
information of the greatest value as regards their potability,
and the examination of water by bacteriological methods

blood heat; also of some sarcinæ and a few micrococci; *B. coli* and *B. Welchii* are usually absent. When, however, the water passes through cultivated lands, or receives sewage, the number of organisms is enormously increased; a large proportion of them liquefy gelatin and develop at blood heat, and *B. coli* and *B. Welchii* appear more or less numerous. Whereas water from shallow wells has a bacterial content nearly as great as the surrounding surface water, that from deep wells, especially in the chalk, is remarkably free from organisms. The following table illustrates the number of organisms that may be met with in water from different sources:

Source	Number of Organisms per cubic centimetre
Freshly fallen snow	34 38
Ice	(very variable) 30 1700
Rain water (Paris)	4 5
Rhone, above Lyons	75
Rhone, below Lyons	800
Rhine, at Mülheim	average about 20,000
Thames, at Hampton (Frankland)	(variable) 2000-90,000
Deep well in the chalk (Kent Company)	3-19
Surface well	1200
Spring water, Reigate (Frankland)	8
Lake of Lucerne	8 50
Loch Katrine (Frankland) . .	74
Filtered water supplied to	
London (Houston)	average rarely exceeds 100
Sewage (Frankland)	26,000,000

The number of bacteria in a natural water varies considerably with its source, at different seasons, and under different climatic conditions.

MANUAL OF BACTERIOLOGY

The following table illustrates the seasonal variation :

Average Number of Microbes per c.c.
(Gelatin at 20°-22° C.) Counted on third day.
Raw Water—749 Samples.

1907-8. Month	River Thames raw water at Hampton. 350 Samples	River Lee raw water at Ponder's End. 280 Samples	New River raw water at Hornsey. 249 Samples
Mean for 1906-7	1680	7253	1099
1907.			
Jan	1803	1861	245
Feb	961	1594	573
Mar	1366	1095	303
Apr	493	854	812
May	[436]	[812]	[241]
June	575	1453	496
July
Aug
Sept
Oct
Nov
Dec

source (river &c.) is in its best condition, so that foul water, in flood time or drought, may be avoided. Moreover, storage alone markedly diminishes the number of organisms, partly by subsidence, partly by lack of aëration, and partly probably owing to the struggle for existence going on among them.

2. *Thickness of Fine Sand in the Filter Beds.*—Efficient sand filtration removes quite 99 per cent. of the organisms originally present. The fine sand only has to be taken into account in estimating the removal of organisms and efficiency of a filter bacteriologically. It probably should form a layer not less than 3 feet to 3 feet 6 inches in thickness. Moreover, a filter bed is not efficient at first, but becomes so when a surface film forms, composed of sedimented particulate matter, and of a zoogeal mass of bacteria and algæ.

3. *The Rate of Filtration.*—The removal of organisms is less perfect when the rate of filtration is increased; this should not exceed about 1.5 gallons per square foot per hour.

4. *The Renewal of the Filter Beds.*—New, or recently cleaned, filter beds allow a greater number of organisms to pass through. The beds must be cleaned from time to time by raking up and clearing away the surface layer of sand, for as time goes on the rate of filtration becomes slower and slower, though the bacterial efficiency of the filter beds does not appear to be reduced by prolonged use. The normal bacterial efficiency seems to be rapidly regained after cleaning—within two or three days.

Besides storage and filtration, sedimentation, in the presence of fine particles, also effects a very marked removal of micro-organisms from water. In Clark's process for the softening of waters possessing a considerable temporary hardness (from the presence of soluble bicarbonate of lime), a suitable proportion of lime-water is added, which

MANUAL OF BACTERIOLOGY

[illegible]

FILTERED WATERS

551

Filtered Waters (Houston, loc. cit.)
AVERAGE NUMBER OF MICROBES PER C.C. (Gelatin at 20°-22° C.) Counted on third day.

Month	New River (1498 Samples)		E. London (Lae) (496 Samples)		E. London (Kempson Park) (408 Samples)		(Tulsa) (633 Samples)		Grand Junction (1279 Samples)		West Middletown (1210 Samples)		S. and Vauxhall (1267 Samples)		Lambeth (634 Samples)		Thames- derived Waters (6511 Samples)		Grand Total (7796 Samples)	
	In.	Ex.	In.	Ex.	In.	Ex.	In.	Ex.	In.	Ex.	In.	Ex.	In.	Ex.	In.	Ex.	In.	Ex.	In.	Ex.
Average, 1906-7	10.1	7.4	27.0	15.9	—	—	11.8	7.5	22.9	10.1	17.1	9.0	37.7	18.6	10.1	8.0	22.5	11.5	10.9	10.8
1907.																				
April	[3.0]	9.9	[7.2]	[7.9]	18.4	10.1	9.9	6.8	80.9	9.9	8.4	8.4	119.6	11.5	4.6	4.6	221.0	8.9	161.4	[7.6]
May	5.9	[8.1]	29.4	14.0	17.3	17.0	11.3	4.0	150.6	11.0	[8.7]	[8.7]	38.59	14.6	9.5	9.5	1118.4	10.1	64.7	8.8
June	4.1	4.1	9.9	9.9	100.8	20.4	5.0	5.0	10.8	8.7	9.8	7.1	13.6	11.2	8.7	8.7	19.5	8.9	18.4	7.8
July	4.3	3.5	59.3	28.0	158.8	48.2	11.7	8.7	1703.7	14.2	17.6	13.4	21.3	11.3	6.7	6.7	440.1	13.0	363.7	11.3
August	4.7	4.7	48.9	22.2	84.0	42.9	5.7	5.7	628.9	14.3	11.0	10.1	20.7	11.6	8.5	8.5	102.0	12.1	119.3	10.9
September	5.6	4.7	27.5	23.8	89.5	40.4	9.3	9.5	150.9	13.8	14.8	9.5	79.5	11.9	6.3	6.3	65.4	12.4	49.2	11.3
October	4.6	3.8	20.8	15.6	80.6	41.3	7.2	7.2	8760.7	10.8	10.6	7.3	[8.3]	[8.2]	3.8	3.8	547.3	10.1	464.6	8.0
November	8.2	6.0	9.5	9.5	69.6	39.8	34.9	8.0	909.4	19.4	6.9	6.9	18.9	12.0	4.5	4.5	249.0	13.4	177.3	11.4
December	19.3	18.4	9.5	9.5	[12.2]	[12.2]	13.9	7.4	176.3	4.9	11.1	8.1	17.1	10.9	30.4	10.6	85.5	8.6	40.9	9.5
1908.																				
January	41.9	12.8	18.2	10.8	65.0	26.5	9.8	9.8	181.4	92.8	34.4	16.0	80.5	17.2	66.7	17.2	86.3	20.9	69.3	17.5
February	6.1	4.9	7.5	7.6	16.3	18.3	4.8	2.5	7.5	7.5	18.9	7.6	48.2	14.4	[3.5]	[3.5]	19.0	8.6	16.7	7.9
March	5.3	5.3	9.8	9.8	18.4	16.3	[2.1]	[2.1]	[7.2]	[4.4]	28.7	11.9	8.4	8.4	4.2	4.2	[12.6]	[7.8]	[11.7]	7.7
Average, 1907-8	9.2	5.7	20.7	13.6	92.7	26.3	10.2	8.3	665.9	11.2	14.8	9.2	63.3	12.7	15.4	8.7	150.9	11.1	131.4	9.9

• Inclusive of all results.
† Exclusive of samples containing 100 or more microbes per c.c.
The highest and the lowest results are indicated by italics and brackets respectively.

MANUAL OF BACTERIOLOGY

in the precipitation of the lime in the added lime-
together with the lime present in the water, in the
a fine precipitate of calcium carbonate with which
nisms are carried down. The addition of alum is
method of clarifying turbid water, and a large
of the organisms present are carried down in the
ate.

tables on pp. 550 and 551 illustrate the influence
of filtration and of storage on the bacterial content
er.

THE BACTERIOLOGICAL EXAMINATION OF WATER.

A bacteriological analysis of water affords valuable
ons as to the purity or otherwise of a water, and,
rly carried out, will indicate a pollution so small
nt as to be incapable of detection by chemical

A specimen of water should be collected in clean
condition and sealed by the best of the means of the

whether the pumps have been in operation, &c., for such may markedly influence the number of bacteria found.

The specimen should then be examined with as little delay as possible, for if allowed to stand for any time a large increase in the number of bacteria may take place. Frankland, for example, found that in distilled water, even at the ordinary temperature, organisms multiply enormously :

Hours	Number of Organisms in 1 c.c.
0	1,078
6	6,028
24	7,262
48	48,100

In water of good quality the organisms are found to multiply much more rapidly during the first few days, after which time they become less and less numerous ; but in very impure water multiplication is slow, and the number much more persistent. It is essential, therefore, if reliable results are to be obtained, for the specimen to be examined at once. If this cannot be done the specimen should be packed in ice ; the cold will then inhibit multiplication to any extent. Special double-chambered metal boxes are made for this purpose : the bottle containing the sample (not less than 60 c.c. ; the writer prefers to have not less than 200 c.c.) is placed in the inner chamber, the outer chamber (which surrounds the inner) being filled with a mixture of ice and sawdust, and the whole is packed in a wooden box with felt lining. Besides the sample packed in ice, a 'Winchester quart' of the water may also be collected for examination for the spores of the *B. Welchii* (*enteritidis sporogenes*).

The routine bacteriological examination of the specimen is usually carried out according to the following scheme (slightly modified) drawn up by a committee of the Royal Institute of Public Health : ¹

¹ *Journ. State Med.* xii., 1904, p. 471.

MANUAL OF BACTERIOLOGY

BACTERIOLOGICAL EXAMINATION OF WATER.

committee recommended that the minimal number of tubes should be :

Enumeration of the bacteria present on a medium incubated at room temperature (18° to 22° C.) Search for *Bacillus coli*, and identification and enumeration of this organism if present.

majority of the committee recommended in addition—

Enumeration of the bacteria present on a medium incubated at blood heat (36° to 38° C.)

Search for and enumeration of streptococci.

was not considered necessary as a routine measure for the *Bacillus Welchii* (*enteritidis sporogenes*), in special or exceptional instances it may be desirable to search for this organism. Unless examined within three hours of collection the sample must be ice-packed.

TO BE EMPLOYED FOR ENUMERATION.—The choice

the ultimate aim in water examination) nutrient gelatin gives a relatively larger number of colonies than distilled-water gelatin, nutrient gelatin should be used when one gelatin only is employed. At the same time, cultures in distilled-water gelatin compared with cultures in nutrient gelatin often give useful indications. Thus with an unpolluted water the number of colonies is usually relatively larger in distilled-water gelatin than in nutrient gelatin; with a polluted water the converse is the case. Therefore the use of *both* gelatins (distilled-water and nutrient) is desirable, sets of plates being made with each medium.

A comparison of the ratio of the number of organisms developing at room temperature to those developing at blood heat also gives useful indications. With a pure water this ratio is generally considerably higher than 10 to 1; with a polluted water this ratio is approached, and frequently becomes 10 to 2, 10 to 3, or even less. The actual number of organisms growing at blood-heat is also of considerable value apart from any question of ratio. Therefore plates of nutrient agar should also be employed and incubated at blood heat.

In certain instances it is true that this ratio may be unreliable. Thus with surface waters, especially in the Tropics (as pointed out by Horrocks), varieties of the *B. fluorescens liquefaciens* and *non-liquefaciens* and *B. liquefaciens* may be abundant and grow well at blood heat.

PREPARATION AND REACTION OF MEDIA FOR ENUMERATION.—(a) *Distilled-water Gelatin*.—Ten per cent. gelatin in distilled water, and brought to a reaction of + 10 (Eyre's scale).

(b) *Nutrient Gelatin*.—Ten per cent. nutrient gelatin, preferably made with meat (beef) infusion and Witte's peptone, and brought to a reaction of + 10 (Eyre's scale).

MANUAL OF BACTERIOLOGY

not weather it may be necessary to increase the age of gelatin to 15 or 20.

For enumeration at blood heat *nutrient agar* should be employed, being prepared with the same constituents as nutrient gelatin, but substituting $1\frac{1}{2}$ per cent. of powdered agar for the gelatin. Reaction + 10.

Distilled-water Agar.—Powdered agar $1\frac{1}{2}$ per cent., dissolved in distilled water, and brought to a reaction of + 10.

On account of the changes which occur in the reaction of the media on keeping, the media employed should preferably be more than three weeks old.

QUANTITIES TO BE PLATED, SIZE OF DISHES, &c.—*Gelatin*.—Ordinary water amounts of 0.2, 0.3, and 0.5 c.c. may be used in Petri dishes of not less than 10 cm. diameter, and should be done in duplicate.

Agar.—Two plates may be made with 0.1 and 0.5 c.c., and should be done in duplicate.

The sample must be thoroughly shaken and mixed in all cases before plating.

In examining an ordinary drinking-water there is no need ever to dilute. As 1000 or 1500 colonies can be counted in a plate, and if the number on a plate should be, owing to crowding, uncountable, *ipso facto* this would be sufficient to condemn without an actual count. Dilution is necessary when dealing with river or other water known to be polluted, and of which an estimate of the number of organisms present is desired. In order to count the colonies if very numerous, ink lines may be drawn across the bottom of the Petri dishes so as to divide them into sectors. Ruled paper discs (Pakes's discs) upon which the dishes are placed can also be obtained. The colonies in the sectors are then much more easily counted; or if the colonies be very numerous and evenly distributed, the number in two or three of the sectors may be counted, and the total number on the plate estimated by calculation.

TEMPERATURE OF INCUBATION.—(a) Room temperature = 18° to 22° C. (gelatin plates). (b) Blood heat = 36° to 38° C. (agar plates).

COUNTING.—Counting to be done with the naked eye, preferably in daylight, any doubtful colony being determined with the aid of a lens or low-power objective.

Time of Counting.—Gelatin plates should be counted at the end of seventy-two hours; but in all cases the plates should be inspected daily, in order that the count may be made earlier should liquefaction render this necessary. The number of liquefying colonies should also be noted.

The blood-heat agar plates should be counted at the end of forty to forty-eight hours.

SEARCH FOR *BACILLUS COLI*.—*Method.*—The committee recommend either (a) the glucose-formate broth method of Pakes, or (b) the bile-salt medium of MacConkey. The writer usually employs the latter.

Incubation anaerobically at 42° C. increases the chances

MANUAL OF BACTERIOLOGY

ess with either medium, and is strongly recom-

neutral-red (Grübler's) glucose broth medium is employed by some.

Parietti method (carbol-hydrochloric acid broth), and of carbohic acid media, are now practically given up.

QUANTITY OF WATER TO BE EXAMINED.—As a routine should be the minimal quantity examined for the of the *Bacillus coli*, quantities from a minimum c. to a maximum of 25 c.c. being added to the tubes re media.

preferable to add the water directly to the tubes of medium, even with the larger amounts, rather than concentrate by filtration through a porcelain filter or brushing method, p. 561). The culture media ended may be diluted with at least an equal volume water without interfering with their cultural es, and large tubes or small flasks may be used for or amounts.

If the medium shows changes (acid + gas) suggestive of the presence of *B. coli*, it is only *presumptive* evidence of the presence of this organism. Occasionally other organisms produce a similar change, e.g. *B. lactis aerogenes*, *B. cloacæ*. Hence the necessity for the isolation and identification of the organism as recommended in the next section.

ISOLATION OF *BACILLUS COLI*, IF PRESENT.—If indications of the presence of the *Bacillus coli* be obtained in the preliminary cultivations, the organism must be isolated and identified.

This may be done by making *surface* cultures on plates (sloping tubes generally suffice) of either (a) litmus lactose agar, reaction + 10; (b) litmus lactose bile-salt agar; (c) Conradi and Drigalski agar; or (d) ordinary nutrient gelatin.

The best medium of all is, probably, the nutrose agar of Conradi and Drigalski (see p. 569). Agar media, incubated at 37° C., have the advantage of saving time.

IDENTIFICATION OF, AND TESTS FOR, THE *BACILLUS COLI*.—Having obtained coli-like colonies on the plates made from the preliminary cultivations of the water, subcultures must be made in order to identify the organism. The following, at least, should be made (see also p. 359):

- (a) Surface agar at 37° C. The abundant growth so obtained enables many subcultures and preparations to be made if required.
- (b) Stab and surface cultures in gelatin. This may be done in the same tube.
- (c) Litmus milk incubated at 37° C.
- (d) Glucose litmus medium.
- (e) Lactose litmus medium.
- (f) Peptone water for indole reaction.

CHARACTERS OF THE TYPICAL *BACILLUS COLI* (according to the committee).—The *Bacillus coli* is a small motile, non-sporing bacillus, growing at 37° C., as well as at room

MANUAL OF BACTERIOLOGY

ture. The motility is well observed in a young culture in a fluid glucose medium. It is decolorized by method of staining. It never liquefies gelatin, and gelatin cultures should be kept for at least ten days in order to exclude a liquefying bacillus. It forms thin surface growths and colonies on gelatin, not motile, growing well to the bottom of the stab (facultative aerobe).

It reduces permanent acidity in milk, which is curdled in seven days at 37° C. It ferments glucose and produces with the production both of acid and of gas.

It generally also forms indole (best obtained in peptone-cultures), gives a thick yellowish-brown growth on potato, (greatly dependent on the character of the potato), reduces (about 50 per cent.) ferments saccharose, changes litmus (Grubler's), and reduces nitrates, and half the amount reduced by it from glucose is absorbable by KOH; these tests, if time and opportunity permit, may be used in addition to the foregoing (see also p. 359).

these tubes gives also a quantitative value to the examination, just as in the case of *B. coli*, and the result obtained should be stated. The streptococci should be isolated (best carried out on Conradi-agar plates), and their characters determined.

According to Houston (*loc. cit.*), faeces contain at least 100,000 streptococci per gram. The type of streptococcus generally present is one forming short chains, producing a uniform turbidity in broth, acid and clot in litmus milk within five days at 37° C., and non-pathogenic for mice. (See table, p. 214.)

BACILLUS WELCHII.—As already stated, it is not essential as a routine procedure to search for the *Bacillus Welchii* (*enteritidis sporogenes*), though in certain instances it may be of advantage to do so. A negative result in such cases is probably of more value than a positive one.

For the isolation of *B. Welchii*, 500 c.c. of the water may be filtered through a Pasteur-Chamberland filter, the deposit suspended in 5 to 6 c.c. of sterile water, and 1 c.c. of the suspension added to each of five to six tubes of sterile milk, which are then heated to 80° C. for ten minutes in a water-bath, and incubated anaerobically at 37° C. for forty-eight hours (filter-brushing method). A better method¹ is to employ large boiling-tubes or small Erlenmeyer flasks, each containing 25 to 50 c.c. of sterile milk. To each tube a quantity of water equal to that of the milk is added, the tubes are then heated in a water-bath to 80° C. for fifteen to twenty minutes, some sterilized oil or melted vaseline is poured on the surface to exclude air, the tubes are cooled in water to 37° C. or thereabouts, and incubated for forty-eight hours at 37° C. Not less than 200 c.c. of the water should be used. The typical change in the milk (see p. 403) indicates the probable presence of the organism. To make sure that the change is due to the *B. Welchii*, and not to the *C. butyricum*, 1 c.c. of the whey per 100 grams of body weight should kill a guinea-pig in forty-eight hours when injected subcutaneously.

¹ B. T. Hewlett, *Trans. Path. Soc. Lond.* lv., 1904, p. 123.

MANUAL OF BACTERIOLOGY

Virulence of a Peptone-water Culture has been suggested as a test of contamination, but in the writer's hands has not given reliable results. If sufficient peptone and salt be added to a measured volume of the water to form a 1 per cent. solution of peptone and a $\frac{1}{4}$ per cent. solution of the latter, the mixture incubated at 37° C. for twenty-four hours and injected intraperitoneally into a guinea-pig, a bad water is stated to kill, whereas a good one does not. The amount to be injected is 2 c.c., and death should ensue within forty-eight hours.

INTERPRETATION OF RESULTS.—The interpretation of the results of the bacterioscopic examination of water is a difficult matter, for which experience is necessary. Just as in chemical analysis, it is hardly possible to lay down an absolute standard, a knowledge of the source and surrounding conditions being of the greatest importance in forming an opinion. The ultimate aim is, of course, the detection of sewage or faecal pollution; the bacterioscopic examination does not give any information as to the suitability

Number of Colonies on the Agar Plates.—As mentioned before (see p. 555), it is the ratio of the number of organisms developing on the agar plates to those developing on the gelatin plates that is of importance. In a pure water this is generally considerably less than 1 to 10—e.g. 1 to 20, 30, or 40—while in an impure water the ratio becomes 1 to 8, 5, 3, or more.

Number of B. coli.—The detection of the *B. coli*, and particularly its enumeration, are regarded by all as perhaps the most important part of water examination. The number of *B. coli* is estimated from the amounts of water that have been added to the tubes of media, which, however, assumes that the organism is regularly distributed throughout the sample, and must, as far as possible, be ensured by thorough mixing. The results generally come out fairly concordantly, though irregularities exceptionally occur which can only be obviated by making duplicate sets of cultures. If the tube to which 0.1 c.c. of the water has been added shows the characteristic changes (according to the medium employed) due to the *B. coli*, and this organism is proved to be present, almost certainly all the other tubes will similarly contain the *B. coli*, and this would indicate that *at least* ten *B. coli* were present in 1 c.c. of the water (in dealing with waters known to be polluted, quantities less than those suggested would be employed). If the 0.1 c.c. tube does not change, but all the others do, this would indicate that *at least* one *B. coli*, but *less than* ten, were present in 1 c.c.; if one of the 10 c.c. tubes and the 25 c.c. tube change, then *B. coli* would be present to the amount of one in 20 c.c.; if none of the tubes changes, there would be less than one *B. coli* in 50 c.c., and so on.

If less than one *B. coli* be present in 50 c.c. of the sample, the water is generally regarded as one of considerable purity. If more than this be present, the interpretation

MANUAL OF BACTERIOLOGY

as much on the source. If this be unknown, Pakes¹ held that waters containing *B. coli* in 20 c.c. or more should be condemned, and that which contains the organism in any quantity between 20 c.c. and 50 c.c. be regarded as suspicious. Savage² came to the following conclusions:

Waters which show no *B. coli* in 50 c.c. are of a high degree of purity, and therefore the proved absence of this organism in small amount, and still better in larger quantities, is of great value.

B. coli should be absent from at least 50 c.c. of spring or deep well water, possibly from greater amounts.

In inland surface waters the presence of *B. coli* in 40, 20, 10, 5, 2 or 1 c.c. means contamination, but not necessarily pollution which it is essential to prevent. It may be due to contamination with the excreta of animals grazing on the water-courses, and is by no means necessarily from sewage or other material containing specific organisms of infection. If

typhoid bacilli have been detected' is of little value. It is on the *general* results of the examination, as detailed in preceding pages, that a conclusion is arrived at respecting the purity or otherwise of a water.

Bacillus Welchii.—This organism being abundantly present in *feces* and sewage, its presence in water has been suggested as an indication of pollution. Its spores, however, are very resistant, and it might, therefore, gain access to the water in ways other than by direct pollution—e.g. in dust—and for this reason the committee did not recommend the search for this organism as a routine procedure. On the other hand, Thresh¹ lays a good deal of stress on it, and the following are standards suggested by him, based on an examination for and detection of *B. coli* and *B. Welchii*.

1. Waters showing the absence of organisms capable of fermenting glucose, and of the *B. Welchii*. These we regard as being free from any evidence of pollution.

2. Waters showing the absence of organisms capable of fermenting glucose, but containing the *B. Welchii*, or its near ally. In the few cases of this kind which have come under our observation we have inferred the absence of sewage pollution, but the possible presence of water derived from fertile soil. This inference has been verified on more than one occasion.

3. Waters containing organisms capable of fermenting glucose, but not lactose, but free from the spores of the *B. Welchii*. These are regarded as unpolluted.

4. Waters differing from No. 3 only in containing spores of the *B. Welchii*. These we regard as free from sewage pollution, but as probably containing soil washings.

5. Waters containing lactose fermenters, none of which belongs to the *Bacillus coli* group, and free from the spores of the *B. Welchii*. These we regard as not being sewage-polluted, but as containing surface water or subsoil washings.

6. Waters resembling No. 5, but containing the spores of the

¹ *Public Health*, 1904.

MANUAL OF BACTERIOLOGY

iii. These waters are usually from a source requiring watching, manurial matter probably being used on the area.

Waters containing organisms of the colon group other than *B. coli*, but no spores of the *B. Welchii*. These we do not consider dangerously polluted, but as probably coming from a source such as that referred to under No. 6.

Waters containing organisms of the colon group other than *B. coli*, and also spores of the *B. Welchii*. Pollution is indicated, but possibly from a source not close at hand. The waters require frequent examination is necessary, especially after rains, as such waters usually sooner or later show more signs of pollution.

Waters containing the true *B. coli*, but no spores of the *B. Welchii*. Such waters are occasionally met with. No opinion should be expressed without an intimate knowledge of the source. It may be such water from a source absolutely free from the possibilities of contamination, but usually subsequent examination has revealed the presence of the spores of the *B. Welchii*. Proximity of manured soil is strongly indicated.

Waters containing the true *B. coli* and spores of the

There can be no doubt of the value of the bacteriological examination of water, but it cannot entirely supplant chemical analysis, which on account of its rapidity and the valuable data it yields will probably always remain an integral part of the examination of potable waters. If the water be pure and uncontaminated, the bacteriological examination will occupy three days; but if contamination be present, though it may be *presumed* in the same time, ten days or a fortnight may be required to convert this presumption into a *certainty*, owing to the length of time necessary for determining the characters of the lactose-fermenting organism present.

MEDIA EMPLOYED FOR THE ISOLATION OF *B. COLI*.

1. *Carbolized Gelatin*.—Ordinary nutrient gelatin with the addition of 0·05 per cent. of phenol. (Hardly used now.)

2. *Bile-salt Peptone Water* (MacConkey and Hill). — The composition of this medium is as follows: Sodium taurocholate 0·5 gram, glucose or lactose 0·5–1·0 gram, peptone 2·0 grams, water 100 c.c. The constituents are dissolved by heating; the mixture is filtered, and after filtration sufficient neutral litmus solution is added to give a distinct colour. The medium is then distributed into Durham's fermentation tubes and sterilized by steaming for twenty minutes on three successive days. The medium may be put up in various sized tubes, a measured volume in each—e.g. 10 c.c., 20 c.c., 25 c.c., &c., according to the quantity of water which is to be added. For the larger quantities the medium may be made double the above strength. The inoculated tubes are incubated at 42° C. for forty-eight hours. The *B. coli* reddens and ferments both the glucose and lactose media, so that gas collects in the fermentation tube.

In the routine examination of water the lactose medium is preferable.

3. *Neutral-red Broth* (Hunter, Makgill, Savage).—The dye known as neutral red (Grübler's) is reduced by the action of the *B. coli*, the colour changing to a canary yellow, accompanied by a green fluorescence. The *B. enteritidis* (Gärtner) also reduces neutral red, but the *B. typhosus* does not do so, nor do streptococci, *B. pyocyaneus*, and *Spirillum cholerae*. Some anaerob

MANUAL OF BACTERIOLOGY

ess a reducing action. Glucose agar or broth (0.5 per glucose) is employed, and to every 10 c.c. of the medium of a 0.5 per cent. aqueous solution of neutral red is Savage recommends the following procedure: 10 c.c. water are added to a 10 c.c. tube of neutral-red broth; 10 c.c. of the water contained in a bottle or flask a 10 c.c. the broth of *quadruple strength* is added. Both are at 37° C., and examined daily up to eight days. If occurs, *B. coli* is almost certainly present in the water; on does not occur, its presence is highly improbable.

Glucose Formate Broth (Pakes).—To ordinary meat 1 per cent. peptone, 0.5 per cent. sodium chloride, 2 per cent. glucose, and 0.4 per cent. sodium formate are added. When the glucose has been dissolved by heating, the medium is neutralized with sodium hydroxide (litmus), and after neutralization 2 c.c. of normal sodium carbonate solution per litre are added; the broth is then steamed for twenty minutes, filtered, and distributed into test-tubes, 10 c.c. in each, which are steamed for twenty minutes on three successive days. These tubes are inoculated with the sample, and incubated anaerobically at 12° C. for twenty-four

6. *Conradi-Drigalski Agar*.—*Mixture A*.—To 1 litre of acid beef broth (p. 46) add :

Witte's peptone	10 grams
Nutrose	10 "
Sodium chloride	5 "

Steam for one hour, and add 25 grams of powdered agar. Steam for three hours, bring to a reaction of + 10, and filter through 'papier Chardin.'

Mixture B.—Boil for a few minutes 100 c.c. of Kubel-Tiemann's litmus solution, add 15 grams of pure powdered lactose, and boil again for a few minutes.

Add *B* to *A*, and to this mixture add 2 c.c. of a hot 10 per cent. solution of anhydrous sodium carbonate and 10 c.c. of a 0.1 per cent. solution of krystal violet. The medium is then tubed, 10 c.c. being placed in each test-tube, and sterilized.

In using the medium it should be employed as *surface plates*. The required number of tubes are melted in a water-bath, and their contents poured out into sterile Petri dishes and allowed to set. These sterile plates are then placed in the warm incubator for an hour or so with the lids slightly tilted at one edge, so that the surface of the medium may dry somewhat. The matter to be plated is sufficiently diluted, and from a few drops to 0.5 c.c. are run on to the surface and spread by means of a glass rod bent into a flattened hook, and sterilized by boiling. On this medium in forty-eight hours *B. coli* forms large red colonies, *B. typhosus* and *B. dysenteriae* small blue colonies, and streptococci small delicate red colonies. Other organisms are to a large extent inhibited from developing.

7. *S.D.S. Rebipelagar* (Houston).—'Rebipelagar' has been much used by Houston¹ for the isolation of *B. coli*. It has the following composition: Agar 20 grams, taurocholate of soda 5 grams, lactose 10 grams, neutral red 4 c.c. of a 1 per cent. solution, peptone 20 grams, water 1 litre. The S.D.S. rebipelagar has the following composition: Agar 20 grams, taurocholate of soda 5 grams, lactose 2.5 grams, neutr 4 c.c. of a 1 per cent. solution, peptone 20 grams, sac 2.5 grams, dulcitol 2.5 grams, salicin 2.5 grams.

¹ *First Rep. on Research Work*, Met. Water Board, 11

MANUAL OF BACTERIOLOGY

ISOLATION OF SPECIFIC ORGANISMS FROM WATER.

Principal disease-producing organisms conveyed by water are *B. typhosus*, *B. paratyphosus*, *B. dysenteriae*, and *Spirillum dysenteriae*.

ISOLATION OF *B. TYPHOSUS*, *B. PARATYPHOSUS*, AND *SPIRILLUM DYSENTERIAE* FROM WATER.—There is very great difficulty in isolating *B. typhosus* from water that has been very copiously polluted with specifically polluted sewage; there is even difficulty in detecting it when the specific pollution has been small in amount. The earlier records of the isolation of *B. typhosus* must be accepted with much scepticism, as the methods of identification were formerly incomplete and unsatisfactory. It is necessary to bear in mind that usually, when water has suffered sewage-pollution, the amount of the pollution is relatively very minute when compared with the bulk of the water supply. Moreover, allowing ten days average incubation period of typhoid fever, another week before the disease comes under notice, and another week before that an epidemic is in progress is recognized, at least a month will have elapsed between the date of infection of the

in a few c.c.'s. Practically, however, a large proportion of the organisms are lost in the process; perhaps they get carried into and remain in the superficial layers of the filter-candle, and for this reason, though sometimes employed, this method has been largely given up.

2. *Concentration*.—W. J. Wilson¹ has devised the following method: The water is placed in one or two Winchester quart bottles, and 10 c.c. of nutrient broth are added for every litre. The bottles are placed in a water-bath maintained at 37°–40° C., and are connected by rubber corks and tubing with a condenser (at a lower level) through which cold water continuously passes, and the tube of the condenser is connected to a large bottle (at a still lower level). This bottle is kept partially exhausted by means of a filter-pump. The water evaporates and is thus concentrated, the evaporated water being condensed and is collected in the exhausted bottle. It requires twenty-one to twenty-two hours to evaporate a litre of water. The water remaining in the bottles, now concentrated to a few c.c.'s, is then plated on Conradi-Drigalski or malachite-green agar.

3. *Chemical Precipitation*.—These methods depend on the formation in the water of a fine, inert precipitate, which entangles and carries down with it a large proportion of the bacteria present. Thus, in the Vallet-Schiuder² method, to 2 litres of the water are added 20 c.c. of a 7.75 per cent. solution of sodium hyposulphite and 20 c.c. of a 10 per cent. solution of lead nitrate. The precipitate is allowed to settle or is centrifugalized off, is dissolved in a small volume of a saturated solution of the hyposulphite, from which plates are made in suitable media. Ficker³ uses ferrous sulphate after making the water faintly alkaline with caustic soda; the ferrous hydrate formed carries down the micro-organisms (this must be a risky procedure, as the typhoid bacillus is very sensitive to caustic alkalies). Iron oxychloride may also be used as the precipitant. H. S. Willson (*loc. cit.*) employs alum. A stock solution of alum is prepared, containing 10 grams per 100 c.c., and of this sufficient is added

¹ *Brit. Med. Journ.* 1907, i. p. 1176.

² *Zeitschr. f. Hyg.* xlii. No. 2, p. 317.

³ *Hyg. Rundschau*, xiv. No. 1, 1904, p. 7.

to the water to obtain 0.5 gram to the litre. After the precipitate of aluminium hydrate has formed, the vessel is well shaken to mix its contents, and the mixture is centrifugalized for fifteen minutes at 2000 revolutions per minute. The clear, supernatant fluid is then syphoned or poured carefully off from the precipitate, and the mass of precipitate in the conical extremity of the tube stirred up with the little fluid (0.5 to 1 c.c.) remaining. The suspension is then plated out on Conradi-Drigalski or malachite-green agar. This seems to be a very promising method.

4. *Serum Agglutination*.—An antityphoid serum—the serum of an animal which has been inoculated several times with the typhoid bacillus, having the power of agglutinating typhoid bacilli—if added to a water, would presumably agglutinate any typhoid bacilli into masses which will sediment or may be centrifugalized off. The method has been used by Schepilewsky, who adds 10 to 20 c.c. of the water to flasks containing 50 c.c. of nutrient broth, to which after three or four days' incubation at 37° C. an addition of the typhoid serum is made, and after standing for some hours and centrifugalizing, the deposit is plated out.

5. *Method of Enrichment*.—The principle of this method is to devise a medium which shall allow of the multiplication of the typhoid bacillus, and at the same time prevent, or at least retard, the growth of *B. coli* and allied forms. Almost all the methods which have been introduced for this purpose fail, inasmuch as, though they inhibit the growth of a great many organisms, they do not inhibit the growth of the *B. coli*, or, if they do, inhibit the *B. typhosus* to a still greater degree. Roth² found that caffeine in broth would retard *B. coli*, but allow *B. typhosus* to multiply. The method has been further elaborated by Hoffmann and Ficker,³ who convert the water itself into a nutrient medium by the addition of 1 per cent. of nutrose, 0.5 per cent. caffeine, and 0.001 per cent. of crystal violet. The mixture is incubated at 37° C. for not more than twelve to thirteen hours, at the end of which time the typhoid bacilli

¹ *Centr. f. Bakt., Orig.* xliii. No. 5, 1903.

² *Hgg. Rundschau*, xliii., 1903, p. 489.

³ *Ibid.* xiv., 1904, p. 1.

should have multiplied to such an extent as to permit of direct isolation by plating, the *B. coli* being inhibited. Many observers have shown, however, that while caffeine may materially help, it cannot be entirely relied on to eliminate *B. coli* and allied forms.

6. *Process of Cambier*.—Cambier¹ has devised a process based on the idea that an actively motile organism will find its way through the pores of a porcelain filter more quickly than feebly or non-motile forms. His procedure is to make use of a special alkaline peptone medium, which is placed in a glass jar. In this is immersed a Pasteur-Chamberland filter-candle half filled with the same solution, to which is added a little of the fluid to be examined, and the whole is incubated at 37° C. Sooner or later growth appears in the fluid outside the candle, and Cambier states that if typhoid bacilli be present they will make their appearance before *B. coli*. In hands other than those of Cambier, however, the method has not proved successful.

7. *Fuchsin Agar* (Endo).—One litre of 3 per cent. nutrient agar is made alkaline with 10 c.c. of 10 per cent. NaOH solution after neutralization. Pure lactose 10 grams and saturated alcoholic fuchsin solution 5 c.c. are added, and after mixing, 25 c.c. of a fresh 10 per cent. solution of sodium sulphite are added. The medium when cold should be colourless. The medium is used as surface plates, and on it typhoid and paratyphoid colonies are colourless, coli colonies are red.

8. *Malachite-green Media*.—Löffler has found that malachite green (No. 120 Hoechst) in the proportion of about 1 in 5000 in media inhibits the growth of *B. coli* while still permitting the growth of *B. typhosus*. The dye may be added either to liquid or to solid media. The medium recommended by Löffler² is composed of 3 per cent. agar made with meat infusion, with 1 per cent. nutrose, and containing in every 100 c.c. 2.2-5 c.c. of a 1 per cent. solution of malachite green. On this medium the *B. typhosus* grows in twenty-four hours as delicate, slightly crinkled colonies, surrounded by a colourless zone (due to alkali formed by the bacilli). Thus it is possible to detect one colony of *B. typhosus* among 800 to 600 colonies of other bacteria. As a medium for 'enriching'—i.e. for specially advancing the

¹ *Rev. d'Hyg.* 1902, p. 64.

² *Deutsch. Med. Woch.* 1906, No. 8.

growth of the *B. typhosus*—Löffler recommends a 15 per cent. gelatin, prepared with beef-juice and peptone, and containing per 100 c.c. 3 c.c. of doubly normal phosphoric acid and 2 c.c. of 2 per cent. malachite-green solution. With the suspected matter, firstly, one series of malachite-gelatin plates is prepared and incubated at 25° C. for twenty to twenty-four hours; secondly, a tube of malachite gelatin is inoculated and incubated at 37° c.c. for twelve to twenty-four hours; from this a second tube is inoculated and incubated at 37° C., and then plated out on malachite gelatin and incubated at 25° C. The colonies of *B. typhosus* are well marked after twenty to twenty-four hours, as large as a pin's head, transparent, highly refractile, light grey and granular. Their shape is circular or oval, and they show characteristic offshoots resembling a bone-corpuscle or the body of an acarus. By using this 15 per cent. gelatin, which can be incubated at 25° C., there is the double advantage of speedy growth and formation of very characteristic colonies.

Houston recommends S.D.S. rehipelagar (p. 569) with the addition of malachite green to the extent of 1 in 5000 (0·2 gram to the litre). On this medium *B. typhosus* forms colourless colonies; most other bacteria do not grow, or appear as blue-black colonies.

CONCLUSION.—The writer would suggest for the isolation of *B. typhosus* from water, (1) concentration of the organism by precipitation with alum (Willson's method) or iron oxy-chloride, followed by plating of the precipitate on Conradi-Drigalski agar, or, better, on malachite-green agar (Löffler's or Houston's, No. 8 above); (2) enrichment by Löffler's method and subsequent plating. In all cases the organism isolated must be examined as to its morphological, cultural and biological characters, and should have its agglutination and Pfeiffer reactions tested with a high-grade typhoid serum. Two organisms which are likely to be mistaken for the *B. typhosus*, unless all tests are applied to them, are the *B. (fecalis) alkaligenes* and *B. (aquatilis) sulcatus*. Both occur in the dejecta and in polluted water and are

very like the *B. typhosus* in morphology, motility, staining, cultural and fermentation reactions, but neither agglutinates with typhoid serum. The *B. alkaligenes* sometimes produces a brownish growth on potato, and it renders litmus milk alkaline. The *B. sulcatus* hardly grows at 37° C. and is almost a strict aërobe, little growth occurring in the depth of a stab. Some varieties of *B. coli* and of atypical *B. coli* agglutinate with typhoid serum, so that a positive agglutination reaction does not necessarily prove that an organism is *B. typhosus*.

THE ISOLATION OF THE CHOLERA BACILLUS FROM WATER.—The detection of Koch's comma bacillus (*Spirillum cholerae*) in water, as in the case of the typhoid bacillus, is a matter of some difficulty, as this organism is rapidly overgrown by the ordinary water bacteria. In the examination of suspected water supplies, the best method to employ for the detection of this organism is to take advantage of the fact, first noted by Dunham, that the cholera spirillum multiplies with great rapidity in alkaline saline peptone solution. The suspected water is examined as follows: To 300-500 c.c. of the water are added 1 per cent. each of pure peptone and common salt; the mixture is made faintly alkaline with sodium carbonate, distributed in a dozen small Erlenmeyer flasks, having a layer not more than an inch deep in each, the flasks are loosely capped with caps of filter-paper, and incubated at 37° C. At intervals of ten, fifteen and twenty hours respectively, hanging-drop and cover-glass preparations are made from the top of the liquid, on which there is often a surface film, and care must be taken not to disturb this; these are then examined microscopically for vibrios and spirilla. At the same time agar plates are prepared, and incubated at blood heat. Any colonies that appear which resemble the cholera spirillum are examined microscopically; if the organisms are comma-shaped, they are at once subcultured into peptone water and other media. The original peptone-water cultures are tested for the indole reaction with pure hydrochloric acid, withdrawing some of the contents of the flasks with a sterile pipette. Any likely spirillum isolated must have its cultural and biological reactions investigated and

MANUAL OF BACTERIOLOGY

for the agglutination and Pfeiffer reactions with a high-cholera serum.

survival of the typhoid and cholera organisms in water, see 411 respectively.

ice-creams may be examined by methods similar to those used the material being first melted at a low temperature. Some of could also be centrifugalized and the deposit examined microscopically for gross contamination.

Infection in typhoid fever and cholera, and perhaps bacillary dysentery, is far more frequently water-borne than conveyed in any other way. It might be supposed that the acid gastric juice would prevent this, and it is so in a large number of instances; but some experiments by Macfadyen¹ showed that, whereas in fasting the stomach to which suspensions in water of the cholera bacteria were administered, living spirilla are present in the intestine, when the vehicle is milk none could be detected, the inference being that the gastric juice is only when food is present, the water passing on to the intestine.

EXAMINATION OF SHELL-FISH.—Shell-fish may come from sewage-polluted layings. The following method may be employed for their examination (after Houston):

The outside of the shells are cleansed by thorough scrubbing and rinsing in tap-water, and a final rinse in sterile water. The fish after cleansing are laid on a sterile towel. The operator then cleanses his hands and opens the shells aseptically with a sterile oyster-knife, care being taken to avoid loss of their contained liquor. The liquor as each fish is opened is poured into a sterile litre cylinder, and the fish is cut up with sterile scissors and added to the liquor in the cylinder. Ten fish should be treated, the volume of fish + liquor noted, and sterile water is then added to make up to 1 litre; 100 c.c. liquid therefore corresponds to one fish. In addition, four dilutions of the liquid are prepared—1 in 10, 1 in 100, 1 in 1000, and 1 in 10,000. With the liquid and dilutions gelatin and agar plate cultivations are prepared for the enumeration of the organisms present. Cultures are also made in litmus lactose bile-salt peptone water and in milk for the enumeration and isolation of *B. coli* and *B. Welchii* respectively, taking 100 c.c. 10 c.c., and 1 c.c. of the liquid, and 1 c.c. of each of the four dilutions; in this way the contents of the fish, ranging from one fish to $\frac{1}{1000000}$ of a fish, are examined. The process and principles involved correspond to those described for water. Houston has suggested for oysters as a lenient standard less than 1000, and as a stringent standard less than 100, *B. coli* per oyster. Even ten *B. coli* per fish should be viewed with suspicion, for Hewlett and others have shown that oysters from pure layings contain no *B. coli*.

Watercress &c. may be examined in a similar manner, 100 grams being weighed out and transferred bit by bit with sterilized forceps and scissors to a flask containing 900 c.c. of sterile water. The flask is shaken vigorously, and the washings examined in a manner similar to that employed for shell-fish.

FILTERS.—Reference has already been made to the removal of organisms in water by sand filtration. With regard to filters for domestic use, few of those in the market are capable of doing more than removing particles of

MANUAL OF BACTERIOLOGY

ed matter, while they allow from 5 to 50 per cent., more, of the bacteria present in the water to be to pass through. Such filters are, of course, useless prevention of disease; in fact, rather favour it, by ering a false sense of security; and when in use for me without cleaning, the water after filtration may ee, bacteriologically and chemically, than before n.

edhead and Wood¹ found that the only filters which pable of completely removing organisms were the -Chamberland, Berkefeld, and Porcelaine d'Amiante. rkefeld, while more rapid in action than the other ter being in use for a few days may allow some ns to appear in the filtrate. This, perhaps, is due o a growth of organisms through the pores of the ndle than to a direct passage. Lunt² found that e ordinary water bacteria, such as the *B. fluorescens* iens, appeared in the filtrate from a Berkefeld

Chamberland. All porcelain filters should be cleaned weekly by well scrubbing with a nail-brush and boiling in water containing some sodium carbonate.

THE BACTERIOLOGICAL EXAMINATION OF WATER-FILTERS.

The large majority of water-filters at present in use are incapable of preventing organisms from being washed through into the filtrate. In order to ascertain whether this is the case with any particular filter, it should be sterilized in the steam-sterilizer, and water containing organisms of known species (*B. prodigiosus*, *B. violaceus*, and *M. agilis* are very suitable) should be passed through it for twenty-four hours. This water and the filter should during this period of the examination be maintained, if conveniently possible, at a temperature below 5° C. This will almost invariably prevent any growth or multiplication of the organisms. Samples should be taken immediately after the filtration has begun, and at intervals during the day, and again at the end of twenty-four hours. If they are all sterile, the filter is capable of preventing organisms from being directly washed through. In the case of filters of very great density or depth of filtering medium, it may be necessary to prolong the period of examination beyond the first day; but most ordinary filters which permit organisms to be washed through do so within the first few hours.

PROTOZOA AND ALGÆ IN WATER.

The examination of water for the minute forms of life other than bacteria, and their enumeration, can be carried out by the Sedgwick-Rafter method.¹ A 6 inch glass funnel is plugged at the bottom of the stem with a perforated rubber cork, over the upper end of which a disc of fine silk bolting cloth, cut by a wad-cutter, is laid. Sharp, clean, dry quartz sand is then poured into the stem of the funnel to the depth of half an inch above the plug. The sand should be of such a size that the grains will pass through a sieve of 60 meshes to the inch, but not through one of 120 meshes. The sand is washed into place and moistened with a little distilled water free from organisms.

¹ Calkin, 23rd Ann. Rep. State Board of Health, Massachusetts, 1

case the supernatant fluid carrying with it the organisms is withdrawn by a pipette from the bottom of the bottle and transferred to the counting glass slide on which a rectangle of glass is cemented, so enclosing exactly 1 mm. thick, so that the preparation is covered with a cover glass of 1 mm. power.¹

THE BACTERIA

Just as in water, the bacteria are found at different times and in different conditions, and in various localities. They are mostly saprophytes, consisting of a number of moulds occurring in large excess, to

It is not easy for micro-organisms to pass through the atmosphere; they are not rising, and cannot be torn from the medium by a strong current. When they are growing must dry in the air as fine dust before they can be carried by the air currents (but see p. 8).

The next day

freed from organisms. Frankland found at Norwich Cathedral at an altitude of 300 feet 7 organisms in two gallons, while on the ground 18 were found; at the Golden Gallery at St. Paul's two gallons of air contained 11 organisms; in St. Paul's churchyard the number was 70. On high mountains organisms are nearly absent from the air, and the same is the case at sea at a distance from land exceeding about 100 miles. Organisms are much fewer in the air of the country than in that of towns. At the entrance-hall, Natural History Museum, South Kensington, Frankland found in the morning 30 organisms; in the afternoon, when many visitors were present, the number had risen to 292, showing the influence of movement. By keeping a volume of air absolutely still, enclosed in a box the walls of which were smeared with glycerin, Tyndall was able to free it completely from particles and organisms. The writer found from 43 to 150 organisms per 10 litres of air in some of the principal streets of London during the daytime.

Gordon,¹ by exposing dishes of neutral-red broth to the air, or by aspirating air through neutral-red broth (p. 567), has been able to detect the presence of the *S. salivarius*, *M. epidermidis*, and scurf micrococcus (p. 207) in air subjected to human contamination. By these tests and by the use of *B. prodigiosus* as an indicator he concludes that particles of saliva are disseminated as far as 40 feet in the act of loud speaking, indicating the possibility of the wide distribution of such pathogenic organisms as the tubercle, plague, and influenza bacilli and the pneumococcus by speaking, and still more so by coughing.

The number of *dust* particles in the air may be enormous. In London Macfadyen and Lunt observed as extremes from 20,000 to nearly 600,000 per c.c. The ratio of micro-organisms to dust particles is therefore a very small one.

¹ *Reps. Med. Off. Loc. Gov. Board for 1902-190*

MANUAL OF BACTERIOLOGY

BACTERIOLOGICAL EXAMINATION OF AIR.

number of methods have been devised for the estimation of number of micro-organisms in the air, of which the following are the chief :

Plate Method.—Melted sterile nutrient gelatin is poured into a sterilized Petri dish, and allowed to set. The plate is then exposed to the air, by removing the lid, for a given time—one, five, ten, or fifteen minutes, &c.—the lid is replaced, and the plate is incubated at 22° C. for some days. The number of colonies of moulds, bacteria, yeasts, &c. is counted, and, having estimated the area of the gelatin plate,¹ the result is expressed as the number of organisms falling per square foot per minute. The results obtained by this method are roughly comparative, but no definite conclusion can be formed from it of the number of organisms present in a given volume of the air.

Jesse's Method.—This is a quantitative method for estimating the number of organisms contained in a given volume of air. The apparatus consists of a glass tube 30 inches long by 1½ inches in diameter. One end of this tube is plugged with a rubber cork through which a glass tube passes, the other end of which is connected with a funnel which is fastened to a

litre of air passes through the tube, and this process is repeated until 5, 10, 15, or 20 litres of air have been drawn through the tube. The rate of flow is controlled by a screw-clamp on the rubber connecting-tube; it should not exceed half a litre per minute. With this rate of flow all the organisms are deposited on the gelatin-coated tube. The aspiration being completed the rubber tube is disconnected and the sheet of rubber replaced over the end of the tube, which is then incubated, and the colonies counted when they have developed.

8. *Petri's Method*.—Petri aspirates the air through a glass tube containing sterilized sand, kept in place by fine wire-gauze wads. When the sample has been taken, the sand is distributed in Petri dishes, and melted sterile gelatin is poured over it and allowed to solidify, plate cultures being thus prepared. The objection to this method is the presence of the opaque particles of sand in the culture medium.



FIG. 70.—FRANKLAND'S TUBE FOR AIR ANALYSIS.

4. *Frankland's Method*.—The air to be examined is aspirated through a tube 5 inches in length and $\frac{1}{4}$ inch in diameter (fig. 70). One end of the tube is open, the other (c) is plugged with cotton-wool. At a distance of 1 inch from the open end the tube is slightly constricted to support a plug of glass-wool (A). At a distance of $2\frac{1}{2}$ inches from this plug the tube is again constricted to support a second plug (B), consisting of glass-wool and finely powdered cane-sugar, supported in front and behind by plugs of glass-wool. Several such tubes having been prepared, they are placed in a tin box and sterilized at 180° C. for three hours, and can then be easily transported without risk of contamination. When required for use, a tube is quickly removed from the box, being handled by the plugged end, which is connected by stout rubber tubing to aspirating flasks such as are used in Hesse's apparatus. The tube is clamped horizontally to a retort stand, and by attaching the second flask to a small hand exhaust-pump, the

water can be syphoned over from the first flask, a corresponding volume of air passing through the tube. When the desired volume of air has been aspirated through the tube, it is disconnected and placed in another sterile tin box. As many tubes as desired can be employed to control one another or to examine the air in different localities and under different conditions.

All the samples having been taken, the tubes are manipulated on returning to the laboratory. The tubes, as before, being handled by the ends only, a file mark is made across the centre of each tube, which is then broken in half and the plugs of glass-wool and sugar shaken, or pushed by means of a sterile wire, into a sterile flask of about 250 c.c. capacity. Into this 10 or 15 c.c. of liquefied sterile nutrient gelatin are then introduced; the sugar dissolves, the glass-wool becomes disintegrated, and a roll-culture is made on the walls of the flask, which is incubated at 22° C., and the colonies counted when they have developed.

5. *Sedgwick and Tucker's Method.*—One of the best and most convenient methods for the bacteriological examination of air. A glass tube of special form is employed (fig. 71); this consists of an expanded portion (A) about 15 cm. long and 4.5 cm. in diameter; one end of this is contracted so as to form a neck 2.5 cm. in diameter and in length; to the other end is fused a glass tube (B C) 15 cm. long and 0.5 cm. in

diameter. The neck of the tube is plugged with cotton-wool, and two cotton-wool—or, better, glass-wool—plugs are inserted in the narrow tube, one at its open end, the other (C) about 6 to 8 cm. from the wide part. The whole is then sterilized. When cool, the narrow part of the tube, from its origin at the wide part down to the first plug (B C), is filled with powdered cane-sugar (No. 50, B.P. gauge) which has been carefully dried and sterilized at 120°–130° C. The tube is again sterilized at 120°–130° for



FIG. 71.— SEDGWICK AND TUCKER'S TUBE FOR AIR ANALYSIS.

two or three hours, the greatest care being taken not to melt the sugar. After sterilization the tube is ready for use. The wool plug is removed from the mouth and a measured volume of air is aspirated through the layer of powdered sugar by means of a small hand air-pump, the volume of air being measured by the displacement of water in a flask. Having taken the sample (5 to 20 litres), the wool plug is replaced in the neck. The powdered sugar is then shaken down into the wide part of the tube (A), and 15 c.c. of melted sterile nutrient gelatin are poured in. The powdered sugar readily dissolves in the melted gelatin, and when solution is complete a roll-culture is made in the tube, just as in Esmarch's method (p. 72). The tube is then placed in an incubator at 20° C., and the colonies are allowed to develop.

In both Frankland's and Sedgwick and Tucker's methods the sugar, after powdering and sifting and before introducing into the tubes, should be thoroughly dried by keeping in the warm incubator for several days with occasional stirring. Unless this be done, the sugar is apt to cake and discolour during sterilization.

SOIL.

The upper layers of soil contain large numbers of organisms, chiefly bacilli. The species are very varied; among pathogenic ones may be named the bacillus of tetanus and of malignant edema. The *B. mycoides* is very abundant, and the varieties of *Proteus*, the hay, and potato bacilli, are common, while the nitrifying forms are of course present, but do not develop on ordinary media.

Below 5 or 6 feet aerobic organisms become scanty, but the anaërobic and thermophilic ones are still met with. The number of organisms present in soil is variable, from 200,000 to 45,000,000 in ordinary earth, while in dirty and busy streets there may be as many as 1,000,000,000, per gram. According to Houston, uncultivated sandy soil averages 100,000, garden soil 1,500,000, and sewage polluted 115,000,000, per gram.

Houston¹ found that in virgin soils the *B. coli*, *B. Welchii*, and streptococci are practically absent, but that in soils polluted with animal excrement by manuring or otherwise the spores of *B. Welchii* are present in great abundance, also *B. coli* and streptococci if the pollution be of recent date.

¹ Rep. Med. Off. Loc. Gov. Board for 1889-1900.

The length of time pathogenic bacteria retain their vitality in buried corpses has been the subject of experiment by Lösenar,¹ who injected cultures into the bodies of pigs, which were then wrapped in linen, placed in wooden coffins, and buried. The conclusions he arrived at were that, provided the soil has good filtering properties, there is practically no chance of the dissemination of a virus.

Klein,² experimenting with the bacilli of diphtheria, cholera, plague, typhoid fever, &c., also found that the vitality and infective power of these organisms passed away in a comparatively short time, in most cases within a month.

On the survival of the typhoid and cholera organisms in soil see also pp. 338 and 411 respectively.

EXAMINATION OF SOIL.

The bacteria in the soil may be examined by adding traces of the soil to sterile nutrient broth, thoroughly crushing and soaking it, and then making plate or roll cultures, aerobic and anaerobic.

To make anything like an accurate quantitative examination is almost impossible. Weighed amounts of the soil, after thorough pulverization in an agate mortar, may be introduced into sterile test-tubes and thoroughly exhausted by repeated washing with sterile water or broth, plate cultivations being made with the washings.

Various boring apparatus have been devised for withdrawing soil from different depths.

SEWAGE.³

Sewage is exceptionally rich in organisms, but the numbers present are variable. Jordan in Massachusetts found an average of 708,000 per cubic centimetre. Laws and Andrewes found from 905,000 to 11,216,000, the latter being the highest number obtained. The number of organisms naturally varies at different seasons and with the amount of dilution. The organisms present are very varied, but moulds, yeasts, and sarcinae only occasionally

¹ *Centr. f. Bakt.* (1^{re} Abt.), xx., 1896, p. 454.

² *Rep. Med. Off. Loc. Gov. Board* for 1898-99, p. 344.

³ See various *Reports to the London County Council* by Clowes, Houston, Laws and Andrewes; Klein, Houston, *Reps. Med. Off. Loc. Gov. Board* for 1897-1904; *Rep. of the Sewage Commission*.

occur. A few micrococci are met with and streptococci are present in considerable numbers, at least 1000 per c.c., but bacilli, especially liquefying forms, largely predominate. The commonest species are the *B. fluorescens liquefaciens* and varieties, several varieties of *Proteus*, the *B. filamentosus*, varieties of the *B. mesentericus*, *B. mycoides*, *B. subtilis*, *B. cloacæ*, and the colon bacillus. The latter numbers from 20,000 to 2,000,000 per c.c., and the other bacilli mentioned number 200,000 to 2,500,000 per c.c. Many anaerobic sporing bacilli are also found, especially the *B. Welchii*, the spores of which number from 30 to 2000 per c.c., averaging 500-600. Bacteria introduced into sewage are probably soon suppressed by the predominant species of the sewage.

The air of well-ventilated sewers differs but little from that of the external air, and the organisms in it contrast with those of sewage by the abundance of moulds. Specific organisms may, however, gain access to it (p. 340).

The powerful liquefying and solvent actions of the bacteria present in sewage have suggested a means of dealing with sewage so as to make use of these properties, and many bacterial systems of sewage disposal have been devised. The principle most widely adopted is to run the sewage into large covered reservoirs (septic tanks), where it remains at rest for twenty-four to forty-eight hours. Here it is under practically anaerobic conditions, and anaerobic bacteria exert their action on the solids, partly dissolving them, partly disintegrating them, with the formation of a sludge which has to be cleared out from time to time. From the septic tanks the sewage passes on to beds composed of broken brick, coke, or some similar material, through which it slowly percolates, and here it is subjected to the action of aerobic organisms which complete the decomposition to such an extent that the effluent does not affect fish life nor putrefy, so that it may be run into a stream without causing a nuisance. Four sets of these aerobic bacterial beds are usually provided, each set being worked in turn for six hours and resting for eighteen hours, during the twenty-four hours. The effluent from such bacterial beds may contain as many bacteria as, or more than, the sewage itself. Pathogenic organisms

MANUAL OF BACTERIOLOGY

esent in it, for Houston found that the *B. pyocyaneus* he beds soon appeared in the effluent.

survival of the typhoid and cholera organisms in sewage see 412 respectively.

MINIATION OF SEWAGE AND SEWAGE EFFLUENTS.

ure a fair average sample, the sewage or effluent should ed in small portions at intervals. The portions are ined through muslin, and dilutions of 1 in 10, 1 in 100, and 1 in 10,000 made with sterile tap-water. These xamined according to the following scheme :

ests	Procedure	Amount of Sewage in c.c.
number of in of spores of s	Gelatin and agar plate cultivations	0.001, 0.0001, 0.00001
	Gelatin plate cultures with material previously heated to 80° C. for ten minutes	1.0, 0.1, 0.01
of spores of thes	Agar plate cultures with material previously	1.0, 0.1, 0.01

MILK.¹

Milk is an admirable nutrient soil for the development and multiplication of micro-organisms, and, though sterile in the udder,² as delivered to the consumer may contain an appalling number of bacteria. In milk as ordinarily supplied there are from one to five million bacteria per c.c., and it frequently contains ten to fifteen millions, with an average of about three to four millions. Hewlett and Barton found an average bacterial content of about 1,500,000 in London milk *as delivered at the railway termini* (the range was from a minimum of 20,000 to a maximum of 8,890,000), but this does not represent the condition of the milk *as delivered to the consumer*, for the bacteria present rapidly multiply in warm weather. Eyre³ in the middle of summer found the following rate of multiplication :

	Microbes per c.c.
Initial content . . .	56,000
After 12 hours . . .	528,000
After 24 hours . . .	20,368,000
After 30 hours . . .	clotted

A similar specimen in the middle of winter gave the following results :

	Microbes per c.c.
Initial content . . .	20,000
After 12 hours . . .	24,000
After 24 hours . . .	48,000
After 30 hours . . .	280,000

In New York, Park estimated the average bacterial content of milk as supplied to the consumer at 1,000,000 per c.c. in winter and 5,000,000 per c.c. during the hot months. Eyre (*loc. cit.*) states that, as the result of his

¹ See Houston, *Rep. to London County Council*, No. 983, 1905; MacConkey, *Journ. of Hygiene*, v., 1905, p. 333; Hewlett and Barton, *ibid.* vii., 1907, p. 22; Swithenbank and Newman, *Bacteriology of Milk*.

² The 'fore' milk may contain organisms which have lodged in the milk-ducts.

³ *Journal of State Medicine*, xii., 1904, p. 728.

MANUAL OF BACTERIOLOGY

ons, the numbers are in London about 3,000,000
000 in December, January, and February, and
0 to 30,000,000 in June to September; smaller
than these always being associated with the
of boric acid or formaldehyde. Even in so-called
milks bacteria are rarely completely absent.
is even richer in bacteria than milk, and averages
00,000, and may contain as many as 30,000,000
s per c.c.¹ Although all the ordinary species may
with, milk has a bacterial flora largely its own,
g many forms producing lactic and butyric acid
ions. Organisms also occur having more or less
ffects, and giving rise to bitter milk, viscid milk,
lactic ferments are mostly non-sporing, the butyric
poring, species. The commonest of the lactic
is the *B. acidi lactici*, which has some similarity to
bacillus (see table, p. 354). Another common lactic
is the *Cidium lactis*, a mycelial form, the colonies

the dirty methods of those handling the milk. In order to render milk wholesome for infants and free from infective organisms under the *present* conditions of supply, two methods may be adopted—sterilization and pasteurization. To ensure sterilization it is necessary to heat the milk to boiling-point for six hours, or to expose it for a shorter period to steam under pressure. Such treatment, however, markedly alters the flavour of the milk, and is said to diminish its nutritive value. If the milk be heated to a temperature not exceeding 70° C., the flavour and nutritive qualities are far less altered, while the pathogenic species are all destroyed. This method is termed 'pasteurization,' and consists in heating the milk to 68° C. for twenty to thirty minutes.¹ Pasteurization destroys 92-99 per cent. of the total organisms present. Behring has advocated the addition of formaldehyde to all milk used for the feeding of children. Another promising method for sterilizing milk is the Budde process,² in which the milk, after the addition of hydrogen peroxide, is heated for three hours to 52°-53° C. All non-sporing organisms are destroyed, and the added hydrogen peroxide is decomposed into H₂O and O.

All milk should be distributed in closed bottles, and pasteurized milk should be consumed within forty-eight hours of treatment.

The thermal death-point of pathogenic organisms in milk is as follows:³

Organism	Temperature.	Period of Exposure
<i>B. tuberculosis</i> . . .	60° C.	20 min.
<i>B. typhosus</i> . . .	60° C.	2 min.
<i>B. diphtherie</i> . . .	60° C.	1 min.
<i>Spir. cholerae</i> . . .	60° C.	1 min.
<i>B. dysenteriae</i> . . .	60° C.	10 min.
<i>M. melitensis</i> . . .	60° C.	20 min.

¹ Macfadyen and Hewlett, *Trans. Brit. Inst. Prev. Med.* i., 1897, p. 82.

² Hewlett, *Lancet*, 1906, i., Jan. 27.

³ Rosenau, Hygienic Lab., Washington, *Bull.* 42, 1908.

MANUAL OF BACTERIOLOGY

thermal death-point of the tubercle bacillus, especially in milk, has been the subject of some controversy (see also p. 288). Woodhead found that an exposure of fifteen minutes at 65° C. was sufficient to destroy the infective properties of tuberculous milk. The Copenhagen Commission, however, considers that pasteurization cannot always be relied upon, and recommends that milk should be heated to 65° C. for ten minutes. The writer found that the vitality of the ordinary non-tuberculous laboratory cultures was destroyed by a temperature of 65° C. acting for ten minutes, and that the infective properties of tuberculous sputum, tested on guinea-pigs, were destroyed by a temperature of 65° C. acting for fifteen minutes in five out of six cases.¹ Woodhead's experiments (First Royal Commission on Tuberculosis) gave irregular results which seem to be explained by the fact that he did not follow Smith's careful work.² This showed that tuberculous milk was rendered non-infective by heating to 60° C. for ten to fifteen minutes, *provided there was no formation of a surface film*. The latter seems to protect the bacilli. Russell and Woodhead³ confirmed Smith's experiments and assert that it is sufficient to heat milk to 60° C. (140° F.) in a closed receptacle for a period of not less than twenty minutes in order to destroy the tubercle bacillus.

(500,000–1,000,000 per c.c.) may often be obtained from quite healthy cows. Blood may also be present transitorily in health (Revis). The presence of squamous epithelial cells indicates desquamation from the teat or udder or from the hand of the milker—i.e. want of cleanliness.¹

There is no doubt that micro-organisms are far more abundant in milk as supplied to the consumer than should be. This arises from the ignorance and carelessness of those charged with the duty of providing and distributing this important article of diet. The udder and teats of the cow and the hands of the milker (who should wear a special dress) should be wiped before milking, and all vessels should be clean and steamed or scalded before use. The milk should be cooled at once, some more efficiently closed vessel than the present form of milk churn adopted, and the milk not stored but forwarded without delay by the railway companies in special refrigerator vans. Distribution in bottles would be a great improvement.

The following might be suggested as a bacteriological standard for milk:² (a) number of organisms not to exceed 1,000,000 per c.c.; (b) absence of excess of leucocytes or of pus cells; (c) *B. coli*, *B. Welchii*, and streptococci should not be present in 1 c.c. or less; (d) the sediment after centrifugalizing should be less than 100 parts per million; (e) the milk as delivered should not have a temperature above 10° C.; (f) absence of pathogenic organisms.

EXAMINATION OF MILK.

Number of Organisms per c.c.—This is carried out by diluting the milk to 1 in 1000—1 in 100,000 with sterile water, or preferably nutrient broth, as a better mixture is obtained. Plates are then made either in gelatin or in distilled water agar (1½ gram

¹ See Eastes, *Brit. Med. Journ.* 1899, ii. p. 1341.

² See 'Rep. of a Committee on Milk Supply,' *Philad. Med. Journ.* Oct. 1900, p. 758; Park, *Journ. of Hygiene*, i., 1901, p. 391; Houston, *loc. cit.*

MANUAL OF BACTERIOLOGY

and agar, distilled water 1 litre, Eastes), or preferably in media.

Coli, B. Welchii, and Streptococci.—These are searched for actively by the methods detailed for 'Water' (pp. 557, 558). Amounts of milk in decreasing decimal order from 1 c.c. to 0.000001 c.c. should be examined.

Tubercle Bacillus.—The detection of these, with the exception of the tubercle bacillus, is difficult and uncertain. In all cases the milk should be centrifugalized and the deposit examined. For the detection of the tubercle bacillus staining methods are almost useless and inoculation must be performed. At least 1 c.c. of the milk should be centrifugalized at 2000 to 2500 revolutions per minute for an hour. As many organisms become trapped in the cream, it is advisable to stop the machine after 1 hour, stir in the cream, and again centrifuge. The fluid is then pipetted off carefully, so as not to disturb the sediment, leaving about 3 c.c. in the tube. The sediment and remaining fluid are then well mixed and about 1 c.c. is inoculated subcutaneously and intra-peritoneally into two guinea-pigs respectively (see also p. 304). For staining, a process of solution of the

4. The *M. pyogenes* and the *Streptococcus pyogenes* may be searched for by means of plate cultures on glycerin agar.

5. *Examination of Sediment.*—The milk is centrifugalized for twenty minutes at 1500 revolutions per minute, and the upper fluid is pipetted or syphoned off. Three smear preparations are then made, each with four drops of the sediment, which are spread evenly over three-fourths of the slide. The slides are air-dried, and may be treated with a mixture of absolute alcohol and ether for ten minutes. One slide is stained with Löffler's blue, another by Gram's method, and a third by the tubercle method. The Löffler's blue specimen gives a general idea of the number of bacteria present, and of the presence of pus cells (polymorphonuclear leucocytes). The number of leucocytic cells in twenty fields should be counted, and if they average more than twenty per field (with a $\frac{1}{16}$ -inch), it is regarded as evidence of pus. This is confirmed if in the Gram specimen numbers of streptococci are found. Confirmatory evidence is obtained if in a fresh specimen (undried) of the sediment mounted in a drop of Gram's iodine solution red blood-cells are seen in addition (Pastes, but see caution above, p. 592). Savage¹ counts the leucocytes with the hæmocytometer, of which the following is a modified method (Revis, *loc. cit.*): Take 5 c.c. of milk and dilute to 20 c.c. with water, centrifuge for ten minutes at 2000 revolutions, break up the cream, rotate again for five minutes, pipette off the fluid, leaving about 1 c.c., dilute this with water to 20 c.c., stir up well and again centrifuge. The water is removed to just above the deposit, four to five drops of a saturated aqueous solution of methylene blue are added, and the deposit is well mixed by blowing through a fine capillary pipette. Stand for fifteen minutes and carefully dilute with water to 1 c.c. The cells are counted in this dilution and the usual calculations made. Two concordant counts must be obtained. The sediment should also be examined fresh with the low powers ($\frac{3}{8}$ and $\frac{1}{2}$ inch) for gross and filth contamination—e.g. hair, straw, sand, vegetable matter, &c. Houston (*loc. cit.*) has devised special tubes for determining the amount of the sediment, and Revis (*loc. cit.*) similarly after treatment with Eau de Javelle.

¹ *Journ. of Hygiene*, vi., 1906, p. 123.

MANUAL OF BACTERIOLOGY

It is not likely to convey any infective disease with the exception of tuberculosis and anthrax. It may be examined by streak and plate cultivations, and by inoculation and feeding experiments. *Tinned meats &c.* frequently contain sporing bacilli of the *B. subtilis* and *mesentericus* groups. They may be examined by aerobic and anaerobic cultures and by inoculating mice. Poisonous ptomaines are occasionally present. *Enteritidis* occurs in meat and causes a form of enteritis (see p. 347). In certain intoxications due to bad food known as 'botulism,' Van Ermengem isolated a bacillus, *Clostridium botulinum*; which occurs as a straight rod, 4-8 μ in length, with rounded ends and terminal spores, feebly motile and not stained by Gram's method. It is strictly anaerobic, liquefies gelatin and ferments glucose, but not lactose. Milk is un-affected.

Bread.—Troitzki states that new bread contains no micro-organisms, but Waldo and Walsh found that such organisms as *Aspergillus* and *Bacillus* are not destroyed by passing through the heat of the baker's oven. Cut bread forms a good nidus for the development of pathogenic organisms.

SAPROPHYTIC ORGANISMS

597

Common Organisms of Air, Water, and Soil

Organism and its Size	Morphology	Motility	Spore formation	Staining	Growth on Agar	Growth on Gelatin	Liquefaction of Gelatin	Growth on Potato	Milk	Alkaline	Habitat	Other Characters, &c.
1. <i>Micrococcus agilis</i> , 0.7-1.0 μ	Coccus, diplo-coccus, tetra-coccus, chains and masses	+	-	+	Coral-pink creamy layer at 20° C.	Coral-pink	+	Coral-pink	-	-	Air, water	Does not grow at 37° C. General turbidity in broth, no film
2. <i>Micrococcus oxidans</i> , 0.8-1.0 μ	Cocci	-	-	+	White shining creamy layer	White porcelain-like	-	White porcelain-like	A	-	Air, water, milk	General turbidity in broth. Stabed by Mactoukey to produce acid and gas in bile-salt lactose media
3. <i>B. filamentifera</i> , 3-5 μ	Anthrax-like	-	+	+	Wavy feathery greyish layer	Grey	++	Grey	A C	A	Sewage, water	Does not grow at 37° C. Strict aerobe. Broth clear with sediment
4. <i>B. mycolides</i> , 3-5 μ	Somewhat anthrax-like	+	+	+	Grey creamy layer	Grey	++	Grey slimy	C a	A	Soil, water	Broth turbid. Colonies on agar plate woolly, tuffed, and mould-like
5. <i>B. myxoderium</i> , 3-5 μ	Large rods and filaments	+	+	+	Grey creamy layer	Grey, thin, yellowish	++	Grey creamy	C a	A	Water	Broth turbid
6. <i>B. mesentericus</i> (vulgaris), 2-4 μ	Slender rods and filaments	+	+	+	Dry grey wrinkled film	Grey with film	+	Grey or dry pinkish, crinkled, abundant	C a	A	Water, soil	The potato bacillus. Strict aerobe. Broth turbid with film. Varieties produce pigment (<i>fusca</i> , brown; <i>ruber</i> , red; <i>niger</i> , black)
7. <i>B. subtilis</i> , 2-4 μ	Slender rods and filaments	+	+	+	Molat, grey, sometimes wrinkled	Grey	++	Grey, lili, thickish	C a	A	Hay, dust, soil	The hay bacillus. Strict aerobe. Spores germinate equatorially. Broth turbid with film
8. <i>B. proteus</i> (<i>f. vulgaris</i>), 2-4 μ	Slender rods, filaments, and threads	+	-	+	Thin, moist, whitish	Grey	++	Slight grey	A C	A G	Soil, sewage, bowel, putrid matter	The <i>B. termo</i> . Occurs in putrefying matter. Colonies on gelatin wavy and motile. Varieties: <i>mirabilis</i> , slow liquefier; <i>Zentseri</i> , non-liquefier
9. <i>B. prodigiosus</i> , 1-2 μ	Short rod, almost bacilli	+	-	-	Thick, creamy, brilliant red	Red or pink	+	Red creamy	A C	A G	Air, water	Grows well at 37° C., but produces no pigment. Broth turbid
10. <i>B. fluorescens liquefaciens</i> , 2-4 μ	Slender rod	+	-	-	Thin, creamy, fluorescent, greenish-yellow	Fluorescent, greenish-yellow	++	Brownish	C a	-	Water, sewage	Stabed by Lehmann & Neumann to be identical with <i>B. pyrogallina</i> , but non-pathogenic (p. 216). <i>B. fluorescens non-liquefaciens</i> similar, but non-liquefying

+ = positive; - = negative; or no change; C = coagulating; A = acid; a = alkaline; G = gas. All the above forms are practically non-pathogenic except *B. proteus* (cystitis, abscesses, diarrhoea), *B. prodigiosus* is pathogenic to guinea-pigs by intraperitoneal inoculation. Chromogenic aerobes, eg. *B. anthracis* and *B. cereus*, form, eg. pink, brown, and numerous other bacilli occur in air, water, and soil.

MANUAL OF BACTERIOLOGY

CHAPTER XXII.

DISINFECTION.

STEAM DISINFECTION — CHEMICAL DISINFECTANTS —
THEORY OF DISINFECTION—METHODS OF DETERMINING DIS-
INFECTANT POWER.

Natural agencies restrain the multiplication of disease germs, but enough survive to determine the persistence of zoonotic diseases, and to call for measures by which communities attempt to cope with them. These measures include isolation, prophylactic inoculation, general improvement in sanitation and nutrition, and disinfection. This present chapter describes the methods by which the former

consists of a portable tank from which paraffin gas oil is driven by a pump through a hose (such as is used for the delivery of oil) to which is attached a pole, consisting of an iron pipe 12 feet long, which is protected by a covering of wood, and to the end of which is attached a cyclone nozzle. The fine spray from the nozzle is ignited, and the resulting fierce flame passed over the surfaces to be disinfected. The thorough wetting with water of all such surfaces would practically abolish danger from fire, and by proper adjustment of the power of the flame might maintain a sufficient germicidal action.

Dry heat may also be used, and forms the basis of some disinfectors (Ransome's), but is not nearly such an efficient means as moist heat. The objections to dry heat are, that to ensure the destruction of bacteria and spores the temperature must be high and the heating prolonged. Koch and Wolhügel found that two hours at 150° C. did not always ensure sterilization, and Gaffky and Löffler state that the spores of some organisms are killed only by exposure to hot air at 140° C. for three hours. Moreover, dry heat has little power of penetration, and it requires many hours for the centre of a mass of bedding, or the like, to attain the temperature requisite for sterilization; while some articles and fabrics are distinctly injured by the prolonged heating. The highest temperature which can be safely adopted for a dry-heat disinfecter is about 120° C., and then if large masses have to be treated the heating has to be continued for from eight to ten hours. A rise of 5° C. above this temperature is sufficient to damage many woollen goods, which enhances the objections to a dry-heat disinfecter, as it is difficult to keep the temperature of a large chamber constant.

For the reasons given above, disinfection by dry heat is often impracticable; on the other hand, *moist heat* is more effective, is found to work well in practice, and is now

MANUAL OF BACTERIOLOGY

y adopted. In the household, for articles which be burnt, brisk boiling for an hour or so will be

m Disinfection.—For public disinfectors, steam pressure—i.e. at a pressure greater than that of the ere—is employed. Steam under pressure has not deleterious action on articles, with the exception er, as dry heat, while its penetrating powers are far By 'saturated steam' is meant steam at the ture at which it can condense, and the temperature condensation point rises as the pressure increases. 'erheated steam' is meant steam at a temperature than that at which it can condense; therefore ated steam has to be cooled down into the state ated steam before condensation ensues. If super-steam is used for disinfection, it loses heat by on, and the rise in temperature of the articles approximately corresponds to the fall in tempera- the steam. With saturated steam, on the other

cylindrical boiler with double walls, forming a jacket, and a door at each end. The chamber is of sufficient size to admit bedding, and is built into the partition wall between two rooms, so that each door opens into a different room. Into one of the rooms the infected articles are conveyed, and are placed in the disinfector as lightly packed as possible; when disinfected they are removed by the opposite door into the other room, thereby avoiding all chance of reinfection. Steam at a pressure of about 20 lbs. is admitted into the jacket and then passes to the inner chamber, the object of the jacket being to warm the chamber, and so prevent condensation. For the same purpose hot air is sometimes injected beforehand to warm the chamber and articles, and after the steam disinfection, can again be injected for drying. The length of time required for disinfection does not exceed a half to one hour.

In Thresh's Disinfector the steam is generated from a saline solution (calcium chloride), which has a boiling-point (105° C.) higher than that of water.

The thermal death-point of a number of organisms in pure culture has been determined by many investigators. Eyre suggests the following as 'standard conditions' for determining thermal death-points:

1. Length of 'time exposure' to be ten minutes.
2. Emulsion to be prepared from 'optimum cultivation.'
3. The vehicle in which culture is suspended to be sterile salt solution or sterile distilled water.
4. Strength of emulsion to correspond to about 1 milligramme of culture per cubic centimetre.
5. Bulk of emulsion to be not less than 3 c.c.
6. Emulsion to be contained in test-tube of 1.5 cm. diameter with walls 1 mm. thick.
7. Emulsion to be exposed to moist heat in a water-bath regulated by a delicate and accurate thermo-regulator.
8. Broth cultivations and agar plates both to be used in determining the death of the bacteria; and the period of observation

MANUAL OF BACTERIOLOGY

cultures to be extended, when necessary, to seven or eight days. The experiments to be repeated at least once.

Thermal death-point to be first roughly determined to within 5° C.

Thermal death-point to be finally determined to within 1° C. and to be defined as that temperature which causes the death of all micro-organisms exposed to it, within the ten minutes standard conditions.

Sunlight is not used directly for disinfection, but indirectly in nature and in our homes may not be an unimportant factor. It has previously been referred to at p. 21. Vaccination, although one of nature's methods of disinfection, is not made use of to any extent by man except in the preservation of many articles of food.

Filtration is a method of disinfection by exclusion, and includes both form of sand filtration and filtration through porous substances, as in the Berkefeld and Pasteur-Chamberland filters. It is made use of for the sterilization of water and other fluids.

depressing effects of the emanations from decomposing organic matter. Such are charcoal, ashes, dry mould, and peat (peat has also a germicidal action). Other deodorants, such as quicklime and chloride of lime, act chemically.

The *germicides and antiseptics* may be considered together, for although many antiseptics are not germicidal, all the germicides in small amounts act as antiseptics. The principal germicides and antiseptics are the halogen elements, chlorine, bromine, and iodine, the mineral acids, a large number of metallic salts, phenol and many coal-tar derivatives, and various organic bodies and essential oils.

Theory of Chemical Disinfection.—The theory of chemical disinfection is not yet fully understood. It is probable, as suggested by Paul and Krönig, that the degree of ionization of a solution may have an important bearing on its disinfecting efficiency.

Paul and Krönig¹ made a number of experiments on the *M. pyogenes*, and spores of anthrax, with a view of determining the effects of various acids, bases, oxidizing agents, and metallic salts on bacteria. The salts of mercury, gold, and silver exert a marked germicidal action, strongest in the case of mercury, while the platinum salts are almost inactive. The efficiency of mercuric chloride is markedly lessened by the presence of sodium chloride or other chlorides. Of the oxidizing agents, nitric, chromic, chloric, and permanganic acids act in the order stated; chlorine has the most powerful action of the halogens. Phenol acts better in a 5 per cent. solution than in higher concentrations, and the efficiency is increased by the addition of sodium chloride, but diminished by the presence of alcohol, and under the most favourable conditions it is not nearly such a powerful germicide as mercuric chloride. Mercuric chloride dissolved in absolute alcohol has little or no efficiency, and the addition of sodium chloride reduces

¹ *Zeitschr. f. physikal. Chem.* 1896, xxi., p. 414.

MANUAL OF BACTERIOLOGY

ty. Organisms in masses are less readily acted on by antiseptics than when they are isolated.

The efficiency of a germicidal salt in solution seems to depend on its dissociation. It is believed that the molecules of the salt in solution are more or less dissociated into electrically electrified atoms or 'ions,' and the greater the number of these the more active will the substance be as a germicide. Taking mercuric chloride, bromide and cyanide, it is found that the ionization of the chloride is greater than that of the bromide, and this is more ionized than the cyanide, and the following results show that the germicidal power of the three is in this order :¹

Solution	Number of colonies which developed	
	After 20 minutes' treatment	After 85 minutes' treatment
1 cc. of 1% HgCl ₂ in 64 litres	7	0
1 cc. of 1% HgBr ₂ " "	34	0
1 cc. of 1% Hg(CN) ₂ in 16 litres	∞	33

by the formula $\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K$, where n_1 and n_2 are the numbers of bacteria surviving after times t_1 and t_2 respectively, and K is a constant. In the case of disinfection of anthrax spores with mercuric chloride, Miss Chick found the mean value of K to be 0.44. In the case of *B. paratyphosus*, however, the course of the disinfection is different unless the culture is very young, and Miss Chick concluded that the older individuals are less resistant than the younger. The progress of heat disinfection apparently follows the same course.

*Factors modifying Disinfectant Action.*¹—The efficiency of a disinfectant liquid partly depends on its concentration. The rate of penetration into bacterial cells decreases as the concentration increases above a certain limit. Most disinfectants yield, therefore, a greater amount of disinfectant energy per gramme-hour in dilute than in strong solutions. In oil, glycerin, or alcohol, disinfectants lose some or most of their activity. Of fats, lanolin alone seems compatible with disinfectant efficiency. Some disinfectants form an emulsion on the addition of water, and their efficiency for a given amount of active material may vary within wide limits according to the manner in which they are emulsified. The temperature at which the organism is exposed to the disinfectant has a considerable influence on the extent or rate of disinfection. Up to the optimum temperature at which the organism to be disinfected grows on the medium in which it is exposed the activity of a disinfectant may fall off as the temperature rises, owing to the increased vigour which the organism derives from the improvement in its conditions in respect of temperature. A relatively small difference of temperature—two or three degrees—may make an appreciable

¹ This section is largely taken from *Applied Bacteriology*, Moor and Hewlett, 1907.

MANUAL OF BACTERIOLOGY

in the activity of the disinfectant, and in the selection of disinfectants the failure to remember this led to serious error. Above the optimum a rise in temperature increases the activity of the disinfectant, and this may go to an enormous extent. The same is sometimes true even at temperatures below the optimum, when the organism is in unfavourable conditions for growth. A mixture of disinfectants in many cases has a more powerful effect than can be produced by either separately (Chamberland). The resistance of bacteria to disinfection by different agencies is extremely variable and is also different. Bacteria of one class may be many times more resistant to one disinfectant than to another when both exert an equal effect on bacteria of another class.

Requirements for an Efficient Disinfectant.—The conditions which should be satisfied by an efficient disinfectant for general use are simple, but not easy to obtain. Because the disinfectant effect depends on the strength of the solution,

should yield a cheap solution or emulsion, not act on metals, and be neither caustic nor toxic. Some disinfectant substances may now be considered more in detail.

Acids.—All acids have disinfectant action, and their relative values are interesting in the respect that for them a general law has been fairly well established by Von Lingelsheim, and confirmed by Boer—namely, that the efficiency varies with the degree of acidity. Solutions of acids not of equal percentage concentration, but of *equal acidity*, have approximately the same disinfectant efficiency, whatever may be the acid, and whether it be inorganic or organic.

The acids have no great practical application in disinfection. That which has been most commonly used is sulphurous acid, applied either direct from burning of sulphur (in which case it will also contain SO_2 if there is sufficient moisture to hold the sulphuric acid in solution) or by the use of the liquefied gas. It produces a slow superficial disinfection of a weak and uncertain character even in laboratory conditions. Such experiments avoid, however, to a far greater extent than is possible in practice the difficulty of diffusion, and the unequal diffusion of sulphurous acid in air and its small power of penetration make it less efficient in practice. To obtain even the poor efficiency which is its maximum possible it is necessary for the air to be damp and the room most carefully sealed, and in these conditions it is often more injurious to the objects under treatment than to the bacteria against which it is directed. One of the most efficient methods of applying sulphurous acid disinfection is by means of the Clayton apparatus. The gas is generated by burning sulphur in a current of air at a high temperature, and contains, in addition to SO_2 , traces of higher oxides of sulphur. It is also a very efficient vermin-killer, destroying rats, cockroaches, bugs, fleas, flies, &c.

MANUAL OF BACTERIOLOGY

Alkalies and Soaps.—The degree of alkalinity of a solution affects, but does not by itself altogether determine, the germicidal power, which is also dependent on the nature of the microbe. The hydrates of thallium, lithium, barium, strontium, potassium, sodium, and ammonia have widely different efficiencies, roughly in the order named. For practical purposes only those of potassium, sodium, and ammonia need be considered.¹ They exhibit notably the characteristic of all disinfectants that they work much more efficiently in hot than in cold solution. It is to the alkalis or alkaline carbonates of potassium and sodium that soaps owe such power as they possess against micro-organisms. The relative efficiency of soaps in disinfection may be understated by the results of *in vitro* experiment on laboratory cultures because the resistance of the microbe itself to disinfection by chemical agents, and, indeed, by other agencies, may be small in comparison with the resistance offered by the spores of

is much to be preferred. Lime is inefficient against the more resistant organisms, and lime-washing cannot be considered a sufficient precaution against them or against infections, such as those of scarlet fever and smallpox, of which the exciting organism is unknown

Halogens.—The disinfectant values of dry chlorine, iodine, and bromine are low. Both in a dry and a damp state chlorine is inconvenient, and the others costly; and the use of halogens is therefore practically confined to solutions, notably 'chloride of lime' (a mixture of calcium hypochlorite, hydrate, and chloride) and hypochlorite of soda (chlorox). These have a powerful effect on laboratory cultures, but in practice need to be used in excess proportionate to the amount of organic matter which may be present. Thus, for instance, a 1 per cent. solution of hypochlorite of soda mixed with an equal volume of urine loses the whole of its available chlorine almost immediately, and becomes inert as a germicide. Where the amount of organic matter is small, and the objects are not likely to be injured, the hypochlorites are among the best of known disinfectants, provided they are used fresh. The slow addition of hydrochloric acid, yielding nascent chlorine, increases the activity of a hypochlorite considerably. Iodine trichloride is a powerful disinfectant, of which the use has been suggested, among other purposes, for the sterilization of water. Nessfield has suggested the use of chlorine for sterilizing water on the large scale, and iodine for the same purpose on the small scale (p. 576).

Other Inorganic Substances.—Solutions of salts of mercury exercise a powerful disinfectant action in proportion to the amount of dissolved metal which they contain. The most commonly used is the perchloride (corrosive sublimate). Apart from its extremely poisonous character, it has the disadvantage of forming with albuminoid substances both insoluble and soluble compounds

of little or no germicidal value, sulphuretted hydrogen converts it into the insoluble and inert sulphide, and it acts on some metals. The addition of acids or salts (e.g. hydrochloric or tartaric acid or sodium or ammonium chloride) prevents or largely reduces the formation of insoluble compounds; but it does not prevent the reactions resulting in soluble substances, it may reduce the germicidal power, and the action of perchloride in the presence of albuminoids is therefore very variable. The reduction in germicidal power by addition of sodium chloride is well seen from the following results (Findlay, *loc. cit.*):

16 litres of solution contained		Number of colonies developing after treat- ment for 6 minutes
1 mole HgCl_2	.	8
1 „ HgCl_2 + 1 mole NaCl	.	32
1 „ HgCl_2 + 2 moles NaCl	.	124
1 „ HgCl_2 + 4 „ NaCl	.	382
1 „ HgCl_2 + 10 „ NaCl	.	1087

Extremely high values were at one time given for the germicidal efficiency of corrosive sublimate. This is now known to have been due to its powerful *inhibitory* action, traces of the substance carried over into the subcultures preventing growth (see p. 617).

The Local Government Board recommended the following solution of corrosive sublimate for disinfecting purposes:

Corrosive sublimate	.	.	.	$\frac{1}{2}$ oz.
Hydrochloric acid	.	.	.	1 oz. fl.
Anilin blue	.	.	.	5 gr.
Water	.	.	.	3 gals.

This forms a solution of 1:900 nearly; it would be preferable to use 1 oz. of corrosive sublimate.

The biniodide is also a powerful disinfectant when dissolved in potassium iodide. It is not affected by albu-

minoids nearly as much as is perchloride, and may be incorporated with soap.

Soluble silver salts are powerful disinfectants, weaker than mercuric chloride, but far less sensitive to albuminoids; in blood serum, for instance, silver nitrate is several times as powerful as corrosive sublimate. They are incompatible with chlorides, except in certain organic combinations, from which silver chloride is only partially precipitated. Silver salts are poisonous, though less so than those of mercury.

Iron and zinc salts have been credited with useful disinfectant action; but, in fact, their value is very small, and no practical account need be taken of them. A very strong antiseptic power has been attributed to copper salts, which, according to some experiments, exercise a sufficient disinfectant action on sporeless organisms, such as the *B. typhosus*, to enable drinking-water to be sterilized from such infections by the small quantity of copper which it dissolves (p. 576).

There is some ground for connecting the disinfectant action of metallic salts with a reducing action on some forms of protoplasm, as pointed out by Loew.

The permanganates have considerable germicidal power when in strongly acid or alkaline solution, but the readiness with which they are affected by organic substances makes them unsuitable for practical use. Peroxides and ozone are open to the same objection, and have less disinfectant power. Hydrogen peroxide is used in the Budde process for sterilizing milk (p. 591), and ozone has been practically applied in the sterilization of water supplies.

Organic Substances.—The methane and the aromatic series furnish the disinfectants which are most important in practice.

Alcohol itself possesses some disinfectant power for sporeless organisms, but only when absolute or in very strong solution.

Formaldehyde is by far the most important of the methane group. It can be applied either as a solution (formalin) or as gas. The gas can be produced by the incomplete combustion or oxidation of methyl alcohol, by the evaporation with or without pressure or spraying of formalin, either alone or mixed with calcium chloride or glycerin, by the de-polymerization by heat of the solid polymer paraformaldehyde, or by mixing this substance with potassium permanganate. A number of apparatus have been designed for the production of formaldehyde gas for disinfection. In any form the gas seems to give little more than superficial disinfection, and to require precautions to ensure diffusion throughout the atmosphere of a room. The conditions desirable for disinfection by formaldehyde gas are saturation of the air with moisture, maintenance of a good room-temperature, sealing of the room, the use of at least 60 grams of formaldehyde per 1000 cubic feet (preferably more, up to 120 grams), and in the case of large rooms mixture of the gas with the air of the room, either mechanically or by the provision of a multiplicity of inlets for the gas into the atmosphere. By the use of a vacuum formaldehyde can be evaporated in a closed chamber at temperatures indifferent to many substances which will not stand steam at 100°, and considerable penetration can be obtained (Defries process). As a spray formalin can be used in any ordinary apparatus.

Of the aromatic series, the number of substances and preparations is extraordinarily large. The standardization of methods of examination will, it is to be hoped, eliminate the less efficient.

The best known is phenol (carbolic acid). Its saturated solution contains 6 to 7 per cent. It is only slightly affected by albuminoids, and generally is stable in the presence of organic matter at ordinary temperatures. Its compounds, when it forms any, have themselves some disinfectant

action. With acids this action is usually greater than that of pure phenol, with alkalis less. Light tends to decompose it, but the efficiency is not affected. It is poisonous and caustic. For practical uses its chief value is as a standard, as its disinfectant value is comparatively low, and for spore-bearing organisms it is practically useless. Like the cresols, its efficiency is greatly increased by the addition up to saturation of common salt or hydrochloric acid. The following results well demonstrate the increased germicidal power of phenol by additions of sodium chloride (Findlay, *loc. cit.*):

Solution	Anthrax spores treated. Number of colonies develop- ing after treatment (days)			
	0	1	3	7
3 per cent. phenol	6900	1890	1260	950
3 " " + 1 per cent. NaCl	5720	1450	1820	960
8 " " + 8 per cent. NaCl	1940	150	50	0

Probably the addition of salt alters the distribution of the phenol between the water and the cells, addition of salt increasing the concentration in the bacterial cells.

'Crude carbolic acid' consists mainly of cresols and higher phenols in proportions largely dependent on the source of the tar from which they are prepared; phenol is nearly absent from it. By themselves the cresols are extremely insoluble in water; in oil or alcohol they have little or no disinfectant value. Cresols are much reduced in efficiency by albuminoids. In saturated salt solution the disinfectant value of crude carbolic acid is greatly increased.

Ordinarily neutral tar oils with no appreciable disinfectant value are left in or mixed with tar distillate, and the saponified product produces an emulsion with water. Innumerable products of this type are made. Their efficiency varies not only with their active ingredients, but also with the character of the emulsions which they form, from about the same as that of phenol to about three times as much.

MANUAL OF BACTERIOLOGY

cially they are known as soluble carbolic acid, cresote, &c. Creolin is a type of numerous preparations of the same character. They are all poisonous and positive to albuminoids. If naphthalene is present in it is deposited in cold weather on standing. Lysol is only a solution of the cresols in fat or linseed oil and is mixed with addition of alcohol. It gives a clear solution with water, having slightly less efficiency on naked skin than cresol, much superior solvency for grease, and equal sensitiveness to albuminoids. A number of proprietary disinfectants of high germicidal power are now to be obtained. Such are cyllin, McDougall's M.O.H. fluid, and kerol. The active agents appear to be oxidized hydrocarbons without phenol or cresol, in emulsion in soaps, &c., and they are comparatively non-toxic. The active principle of cyllin is an oxidized hydrocarbon, with a di-phenyl nucleus in place of the single phenyl nucleus in carbolic acid: it is insoluble in water, hence

Carbolic-acid Coefficients obtained by the Rideal-Walker Method¹ (p. 617).

Disinfectant	Observer	Date of Experiment	Organism	Carbolic acid Coefficient (Carbolic Acid = 1)
Absolute alcohol	Fowler	8.05	<i>B. typhosus</i>	0.03
Boric acid	Walker	10.01	"	0(?)
Chinosol	Fowler	11.03	"	0.15
Chlorox	Fowler	1.04	"	21.0
" (with 50 per cent. urine)	Walker	7.06	"	8.0
Copper sulphate	"	6.04	"	0.01
Cyllin*	Fowler	11.06	"	14.0
" (with 50 per cent. urine)	"	5.06	"	11.0
Cyllin	Klein	5.05	<i>M. pyogenes</i>	9.3
"	Simpson and Hewlett	6.06	<i>B. pestis</i>	34.0
Formalin	Fowler	3.05	<i>B. typhosus</i>	0.7
Hydrochloric acid . . .	Walker	2.05	"	11.0
Izal*	Fowler	3.06	"	11.0
Kerol*	"	9.06	"	12.0
" (with 50 per cent. urine)	"	8.06	"	8.5
Little's phenyle	"	5.01	"	2.0
Lysol	"	2.06	"	2.5
Mercuric chloride . . .	"	8.05	"	1000.0
"	Walker	8.05	"	400.0
Potass. permanganate .	Fowler	8.05	"	42.0
" " (with 3 per cent. organic matter) .	Walker	1.07	"	1.0
Zinc chloride	"	1.06	"	0.15

* The germicidal efficiency of these substances has been increased since the date of the experiments recorded.

Iodoform is valuable for dusting wounds, though its penetrating odour is objectionable, and has led to the introduction of many substitutes. Its value as an antiseptic has been greatly discussed; micro-organisms will develop in nutrient media containing a considerable proportion, but probably when in contact with living cells a decomposition is effected, free iodine being liberated, hence its value.

The Essential Oils, *Peppermint*, *Mustard*, *Cloves*, *Thymol* and *Menthol* are powerfully antiseptic.

¹ Fowler, *Journ. Roy. Army Med. Corps*, July 1907.

MANUAL OF BACTERIOLOGY

useless to add a small quantity of disinfectant to a large amount of fluid or solid; the disinfectant must be added in sufficient amount so that the mixture contains the minimum percentage which has been found by experiment to be efficient. For this reason the attempt to disinfect sewers, sewage, streets, &c., with relatively small quantities of disinfectants is useless, and the quantity so wasted would be far better employed in providing water for flushing purposes.

In medical practice, while antiseptics can be applied locally to the skin and, to some extent, for disinfecting the alimentary tract, no substance has yet been discovered which can be absorbed with safety to such a degree as to saturate the body and exert a general germicidal action in infective diseases.

In surgical practice no unbiased observer can doubt the value of antiseptic treatment, but many so-called 'antiseptic operations' are marred by faults of omission and commission and render them far from being perfectly antiseptic. There is still some controversy between the advocates of 'antiseptic' and 'aseptic' surgery. Undoubtedly antiseptics do diminish the vitality, and therefore the reparative power, of the tissues.

for a given time. After treatment, the threads are thoroughly washed with distilled water to remove the antiseptic, and sown on the surface of agar or other suitable culture medium. If no growth occurs the organisms are assumed to have been destroyed. As a matter of fact, however, it is extremely difficult to get rid of the last traces of the antiseptic, which may inhibit growth although the organisms may yet be alive, a fallacy which caused an exaggerated value to be assigned to many substances—for example, corrosive sublimate. The thread method may still be employed, but after treatment the threads should be sown in bouillon, or, better still, if pathogenic organisms be the subject of experiment, inoculated into a susceptible animal.

In experiments with corrosive sublimate by whatever method, the last traces of the substance must be converted into the inert sulphide by treatment with sulphuretted hydrogen.

2. *Garnet Method.*—Small garnets the size of a pea are sterilized, soaked in a suspension or a broth culture of the organism, removed and dried. The garnets with the organisms attached are then soaked in solutions of the disinfectant of known strengths for various periods of time, they are then removed from the solution, well washed with sterile water, and finally placed in tubes of broth.

3. *Rideal-Walker or Drop Method.*—Moor first suggested that the germicidal efficiency of a disinfectant might be compared with that of a standard solution of carbolic acid, which has a definite composition, is stable, and can be accurately standardized, and Rideal and Walker devised an ingenious and simple method for carrying this out. A special test-tube rack is very convenient (fig. 72) in which the lower tier has five holes which hold three or four tubes containing solutions of decreasing strengths of the disinfectant to be tested, and two tubes or one tube containing standard carbolic acid solution of known strength for comparison. The upper tier has thirty holes in two rows, and spaced into six sets of five holes each. These hold tubes of sterile nutrient broth which are numbered from 1 to 30. The test is usually made with a broth culture of *B. typhosus*, but other organisms may be employed. The process is as follows: The five tubes in the lower tier each contain 3 c.c. of the disinfectant and carbolic solutions. Into each in succession, at

intervals of half a minute three drops of the typhoid broth culture are added with a pipette. Half a minute after the *last* tube has been inseminated, a loopful is taken from the *first* tube and inseminated into the first broth tube, and this process is repeated at half-minute intervals until all the broth tubes have been inoculated. The inoculated broth tubes are then incubated at 37° C. for three days, and the occurrence or not of growth is taken as

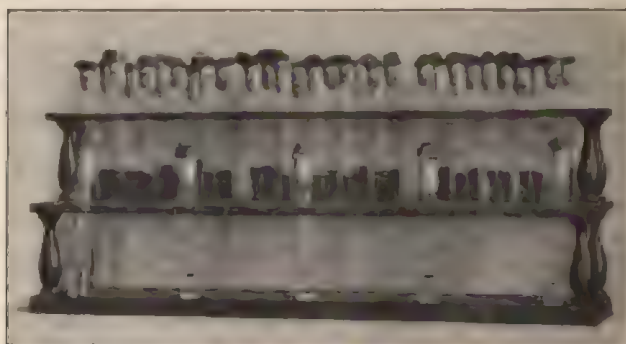


FIG. 72.—TEST TUBE RACK WITH TEST TUBES ARRANGED FOR THE RIDGEL-WALKER METHOD OF TESTING DISINFECTANTS.

indicating the killing or non-killing of the organism respectively. Obviously the first set of five broth tubes inoculated are subcultures in which the organism has been acted upon by the disinfectant and carbolic solutions for 2½ minutes, the second set for five minutes, and so on. The results (taken from an actual test) may be charted as follows :

B. typhosus, 24-hour broth culture at 37° C.
Room-temperature, 60° F.

Disinfectant	Dilution	Time Culture exposed to action of Disinfectant (in minutes).						Subcultures	
		2½	5	7½	10	12½	15	Period of Incubation	Temperature
X	1-1400	+	•	•	•	•	•	3 days	37° C.
X	1-1500	+	+	•	•	•	•		
X	1-1600	+	+	+	•	•	•		
X	1-1700	+	+	+	+	•	•		
Carbolic	1-100	+	+	+	•	•	•		

+ = growth in the subcultures.

• = no growth in the subcultures.

From this it will be seen that the disinfectant X in a solution of 1 in 1600 kills in the same time ($7\frac{1}{2}$ minutes) as carbolic 1 in 100. This result is expressed as a coefficient obtained by dividing the strength of disinfectant by the strength of carbolic which each kills in the same time: in the present instance the co-efficient is $\frac{1600}{100} = 16.0$, and this figure is known as the 'Carbolic-acid Coefficient.'

Precautions to be taken in carrying out the Test. (1) The culture should be a broth one about twenty to twenty-four hours old, and should be free from clumps; this may be attained by filtration through paper.

(2) The carbolic acid should be kept in the form of a 5 per cent. aqueous solution standardized by the bromine method. Failing this, the solutions may be made with the *Acidum carbolicum liquefactum* of the Pharmacopœia, which contains 100 parts of phenol in 110, but is not absolutely constant in composition.

(3) All measures, pipettes, and test-tubes used for making dilutions should be sterile.

(4) The dilutions of the disinfectant and carbolic should be made with sterile distilled water.

(5) The broth used for culturing and subculturing should have the following composition:

Lemco	20 grams
Peptone	20 grams
Salt	10 grams
Water	1000 c.c.

The medium should be standardized to a reaction of +10 (Eyre's scale).

(6) The loop used for subculturing should have an internal diameter of 3 mm., and be made with platinum wire of 27-28 B.W.G.

(7) Growths in the subcultures should be obtained in those taken at not less than two and preferably at three of the time intervals ($2\frac{1}{2}$, 5, and $7\frac{1}{2}$ minutes) from both the disinfectant and the carbolic solutions which correspond.

(8) The temperature at which the determination is made should be noted, and the strength of carbolic varied accordingly

MANUAL OF BACTERIOLOGY

or 56°-62° F., 1-110 for 62°-67° F., and 1-120 for 67°-72° F. for *B. typhosus*), or the determination may be made at standard temperature (e.g. 20° C.) by warming (or cooling) the testant and carbolic tubes in a water-bath.

When the organism does not form a uniform culture in suspension of an agar or other culture must be made in water and filtered. Sub-culturing in some cases (e.g. with *B. typhosus*) must be made on agar or other suitable culture

This method is an admirable one for determining the relative effects of disinfectants on *naked* organisms in the *absence* of organic matter. But in practice disinfection is almost always effected in the presence of organic matter, and various attempts have been made with a view of introducing this into the test, for the presence of organic matter may greatly alter the carbolic-acid coefficient of many disinfectants (see table, p. 611, and table, p. 615). Among the substances suggested are: faeces, 2 per cent. suspension of dried and sterilized milk. Kenwood and Hewlett found that the presence of faeces reduced the carbolic-acid coefficient of some pro-

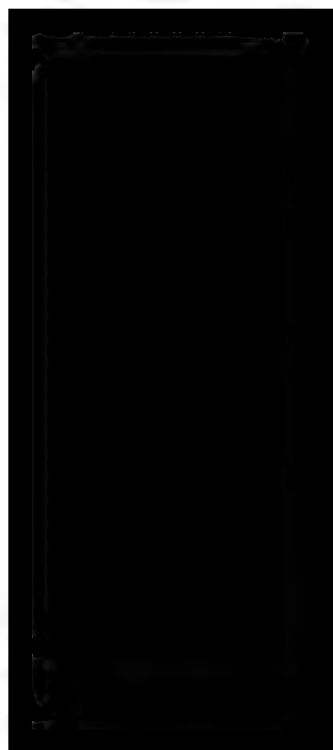
FRENCH WEIGHTS AND MEASURES AND THEIR ENGLISH EQUIVALENTS

1 μ (micron)	=	0.001 millimetre ($\frac{1}{254000}$ inch, nearly).
1 millimetre	=	0.04 ($\frac{1}{25}$) inch.
25 millimetres	=	1 inch.
1 centimetre	=	0.39 inch.
2.5 centimetres	=	1 inch.
5 centimetres	=	2 inches.
1 gram	=	15 $\frac{1}{2}$ (15.432) grains.
4 grams	=	1 drachm (apothecaries'), nearly.
28 grams	=	1 ounce (avoirdupois), nearly.
1 kilogram	=	2.2 pounds (avoirdupois).
0.5 kilogram	=	1 pound (avoirdupois), nearly.
1 cubic centimetre	=	16 minims, nearly (16.23 minims).
3 $\frac{1}{2}$ cubic centimetres	=	1 fluid drachm, nearly.
28 cubic centimetres	=	1 fluid ounce, nearly.
568 cubic centimetres	=	1 pint ($\frac{1}{2}$ litre).
1 litre	=	1 $\frac{1}{2}$ pints, or 35 fluid ounces, nearly.

SOLUBILITIES

AMOUNT OF SUBSTANCE CONTAINED IN 10 C.C. OF A SATURATED SOLUTION

Alcoholic solution of methylene blue	0.068 gram.
Aqueous solution of methylene blue	0.664 gram.
Alcoholic solution of gentian violet	0.442 gram.
Aqueous solution of gentian violet	0.175 gram.
Alcoholic solution of fuchsin	0.292 gram.
Aqueous solution of fuchsin	0.006 gram.
Aqueous solution of corrosive sublimate	0.507 gram.



INDEX.

- ABERRATION, 130
- Abiogenesis, 4
- Abcesses, multiple, 203
- Achalme's bacillus, 530
- Acid alcohol in Gram's method, 95
- Acid-fast organisms, 276
 - in milk, &c., 316
- Actinomyces, cultivation, 428
 - inoculation of, 430
 - varieties, 431
- Actinomycosis, 426
 - clinical examination, 431
 - human, 427
 - in cattle, 426
 - spread of, 430
 - staining of, 431
- Aërobie organisms, 19
- Agar, 50. See Culture Media
- Agglutination, 168
- Aggressins, 162
- Air, bacteriology of, 580
 - examination of, 582
 - of sewers, 587
- Air passages, organisms of, 543
- Air-pump, 41
- Alcohol, absolute, 77
 - formation of, 32, 361, 447
 - for fixing, 76
 - and ether for fixing, 87
 - as an antiseptic, 341, 611
 - methylated, 76
- Alessi's experiments, 340
- Alexins, 158, 180, 183
- Algae, 8
 - destruction of, 576
 - in water, 579
- Amœba coli, 460
 - diagnosis of, 462
- Amœbe, intestinal, 460
- Ammonia not pyogenic, 202
 - production of, 28, 30
- ANAEROBIC CULTURES, 62
 - stab, 62
 - in nitrogen, 64
 - Buchner's tubes, 64
 - in vacuo, 63
 - in hydrogen, 65
 - in formate broth, 68
 - in sulphindigotate broth, 68
 - Dean's method, 68
 - Fränkel's method, 65
 - Hamilton's method, 63
 - writer's method, 67
 - plate, 73
- Anaërobie organisms, 19, 395
- Analysis of yeasts, 439
- Anaphylaxis, 199
- Angina, Vincent's, 272
- Anilin dyes as disinfectants, 614
 - stains, 89
 - water, 90
- Animals, dissection of, 115
 - inoculation of, 113
- Anthraxis, bacillus, 229
- Anthrax, 228
 - bacillus of, 229
 - diagnosis of, 238
 - occurrence of, 232
 - serum for, 237
 - spread of, 234
 - symptomatic, 406
 - vaccine, 237
- Anti-bodies, 139
- Antigen, 139
 - test, 535
- Antiseptic action, conditions modi-
 - fying, 605
 - power, determination of, 616
 - treatment, 616
- Antiseptics, 602 616
 - definition of, 602
- Anti-sera, 156

ANAL OF BACTERIOLOGY

237	<i>Bacillus cloacae</i> , 365
	coli, 355
	communis, 356
	communis, 350
	immobilis, 235
508	coryzae, 274
	diphtheriae, 242
48	columbarum, 274
211	diphtheroid, 268, 274
	dysenteriae, 326, 331
	enteritidis, 327, 347, 507, 596
156	isocardinus, 327
	sporogenes, 402, 405
18	faecalis alkalygines, 574
5	filamentosus, 587, 597
	fluorescens liquefaciens, 34, 216, 555, 587, 597
ed, 187	fluorescens non-liquefaciens, 597
	fusiformis, 273
	grass, 318
30	icteroides, 351, 541
1	influenzae, 392, 529
medium, 53	lactis aerogenes, 364, 590
lium, 449	leprae, 309
	mallei, 318
ing, 442	megaterium, 597
6	mesentericus, 510, 587, 597
	mist, 316
	mucosus capsulatus, 235
	murisepticus, 382
	mycoides, 28, 585, 587, 597
	neapolitanus, 364
	of Achalmes, 402
	of black quarter, 407
	of ... 510

- Bacillus** of swine fever, 350
 of swine plague, 382
 of symptomatic anthrax, 407
 of syphilis, 314, 532
 of xerosis, 273
 Oppler-Boss, 527
 paracoli, 327, 348
 paratyphosus, 327, 347, 348
 perfringens, 402
 pestis, 367
 pneumoniae, 235, 384, 389, 394
 prodigiosus, 597
 proteus, 23, 28, 217, 521, 585, 587, 597
 pseudo-diphtheriae, 263
 pseudo-dysenteriae, 327, 352
 pseudo-tuberculosis, 308, 370, 380
 psittacosis, 351
 putrificus coli, 544
 pyocyaneus, 214, 518, 522, 588
 pyogenes fetidus, 362
 smegmatis, 313
 subtilis, 587, 597
 suicidus, 350
 suispestifer, 370, 382
 tetani, 396, 585
 timothy grass, 316
 tuberculosis, 379
 typhosus, 327
 typhimurium, 327, 347, 351
 typhosimilis, 327
 vagine, 545
 violaceus, 34, 579
 Welchii, 402, 561
 X., 540
 xerosis, 273
- Bacteria**, action on artificial sugars, 20
 classification of, 14
 conditions of life of, 17
 effect of electricity on, 22
 effect of light on, 21
 effect of pressure on, 21
 influence of chemical agents on, 19
 influence of oxygen on, 19
 influence of radium on, 22
 influence of temperature on, 18
 nutrition of, 18
 selective action of, 20
- Bacteria**, structure of, 9 13
 study of, 57, 110 *et seq.*
 thermophilic, 18, 585
 vitality of buried, 586
- Bacterial** poisons, 136
 products, 22
- Bacteriological** diagnosis. *See* Examinations
 microscope, 123
- Bacteriolysis**, 157
- Bacteriotropines**, 199
- Bacterium**, definition of, 16
 species of. *See* Bacillus
 termo, 28, 597
- Bacteroids**, 31
- Balantidium coli**, 475, 522
- Basic** products, 35
- Beale's Carmine**, 93
- Bell-jars**, 41
- Beri Beri**, 518
- Berkefeld** filter, 42, 578
- Bird-pox**, 185, 517
- Bismarck brown**, 91, 270
- Black** leg, 406
- Black** quarter, 406
- Blackwater** fever, 490
- Blastomycetes**, 437
 classification, 437
 examination, 442, 444
 fermentative, 440
 identification, 439
 pathogenic, 442
 spores of, 439
- Blastomycetic dermatitis**, 444
- Bleeding** animals, 116
- Blood** films, 87, 490
- Blood**, germicidal action of, 179, 189
 parasites of birds, &c., 493
 serum, 52, 53
 to obtain, 52, 53, 116
- Blood-agar**, 54, 467
- Blue** pus, 214
- Boils**, 205
- Bordet-Durham** reaction, 169
- Boric** acid, 615
- Botulismus**, 596
- Bread**, 596
- Bromine**, 609
- Bronebitis**, 390, 394, 450
- Broneho-pneumonia**, 383, 387
- Broth**, 47. *See* Culture Media
- Brownian** movement, 121
- Bubonic** plague, 366. *See* Plague

MANUAL OF BACTERIOLOGY

- tube, 64
- process, 591
- , 473
- 96
- ul-fast bacilli in, 316
- mination of, 596
- mixture, 572
- est, 411
- glycerate, fermentation of,
- 08, 510, 527
- oria, 526
- a-rubber, 44
- d bacilli, 285
- f bacteria, 10
- ining, 102
- ehsin, 90
- atin, 567
- ethylene blue, 89
- onin blue, 90
- oid, 612
- ide, 613
- efficient, 619
- s, 200
- n, 185
- 12, 185, 93
- Cholera, spirillum, in oysters, 416
 - in soil, 411
 - in water, 411
 - isolation from water, 575
 - pathogenesis, 412
 - phosphorescence, 413
 - toxins, 416
 - vaccine, 418
- Cirrhosis, hepatic, 362
- Cladothrix dichotoma, 436
- Classification of bacteria, 14
- Clearing, 99
- Clinical diagnoses. *See* Examinations
- Clostridium butyricum, 405, 407
 - Chauvai, 407
- Clove oil as an antiseptic, 615
 - as a clearing agent, 99
- Coccidial disease in man, 478
- Coccidium oviforme, 476
- Cold, effect on bacteria, 18
- Coley's fluid, 227
- Colitis, 522
- Collodion sacs, 113
- Colon bacillus, 355
 - distinction from typhoid, 359
 - in suspension, 202

- Corrosive sublimate as a disinfectant, 609, 610, 617
for fixing, 77, 512
- Cover-glass specimens, 84
of blood, 87, 490
staining, 97
- Cream, 590
- Creolin, 614
- Creosote, 614
- Cresol, 613
- Cribridia, 466
- Croup, 240
- Cultures, anaërobic, 62
hanging-drop, 120
plate, 68, 73
preserving, 107
roll, 72
shake, 74
vitality of, 112
- CULTURE MEDIA:—
- Agar-agar, 50
Blood, 54, 467
Conradi-Dripalski, 569
distilled water, 556, 593
fuchsin, 573
glucose, 51
glycerin, 51
hemoglobin, 54
litmus, 51
malachite green, 573
maltose, 453
nasgar, 219
potato blood, 529
rehipelagar, 569
serum, 53
- Alkali-albumin, 54
- Ascitic fluid, 53, 219
- Beer-wort, 49
- Bile (for typhoid), 345
- Bile-salt, 567, 568
- Broth, acid beef, 46
ascitic fluid, 53, 219
formate, 74, 568
glucose, 49
glycerin beef, 47
lemco, 48, 619
peptone beef, 47
sulphindigotate, 74
veal, 47
- Blood-serum, 52
fluid, 53
Löffler's, 53
- Eggs, 54
- CULTURE MEDIA—*cont.*:
Endo's, 573
Gelatin, 49
beer-wort, 49
carbolic, 567
distilled water, 555
glucose, 49
Hiss's, 268
Hydrocele fluid, 53
Litmus, 51
Löffler's (for typhoid), 573
Malachite green, 326, 573
Milk, 51
Neutral red, 567
Nitric and nitrous, 29
Parietti's solution, 558
Pasteur's fluid, 55
Peptone water, 48
Dunham's, 48
Petruschky's, 360
Potato, 51
glycerin, 280
Proskauer-Capaldi, 359
Standard, 56
Uchinsky's fluid, 55
Whey, litmus, 360
- Cystitis, 362, 597
- Cytases, 183
- Cytoryctes luis, 532
variola, 514
- Cytotoxins, 187
- DANVIR bacillus, 327, 351, 366
effect, 153
rat virus, 351
- Deneko's spirillum, 424
- Dengue, 520
- Deodorants, 602
- Dermatitis, blastomycetic, 444
bullous, 523
- Desiccation as a disinfectant, 602
influence of, 18
- Diagnosis, bacteriological or clinical.
See Examinations
- Diarrhoea of infants, 351, 521, 590
- Dilution method, 68
- Diphtheria, 240
etiology of, 241
antitoxin, 246, 255, 260
standardization, 257
unit of, 259
value of, 260

MANUAL OF BACTERIOLOGY

- a, associated organisms, 247
- agnosis of, 245, 268
- alae of, 246
- milk, 252, 594
- allus, 242
- acid formation, 244
- fermentation reactions, 268
- solation of, 242
- anthogenic action, 249
- ersistence of, 245
- seudo, 263
- hread forms, 243, 271
- oxins, 253
- arieties, 242, 247
- embrane, 249
- ults of examinations for, 248
- lower animals, 251
- calves, 275
- pigeons, 274
- ile roup, 275
- ritis, 250
- alysis, 250, 251
- id bacilli, 250, 263, 274
- ns crassus, 220
- ns, 221
- ECZEMA, 522
- Effluents, sewage, 587, 588
- Egg cultures, 54
- Ehrlich-Biondi stain, 92
- Electricity, effect of, on bacteria, 22
- Embedding, gum, 78
 - paraffin, 80
- Empyema, 330, 388
- Endo's fuchsin agar, 573
- Endocarditis, infective, 205, 210, 222, 387, 531
- Endotoxins, 136
- Enrichment methods, 572, 573
- Entamoeba, 460
- Enteritis, 362
 - fowl, 422
- Enumeration of organisms, 71, 198
- Enzymes, 32
- Eosin, 91
- Eppinger's streptothrix, 431
- Erysipelas, 210
- Esmarch's roll cultures, 72
- Ether and alcohol for fixing, 87
 - freezing, 79
- Evaporation, 41
- EXAMINATIONS, BACTERIOLOGICAL, AND

EXAMINATIONS, BACTERIOLOGICAL, AND

CLINICAL DIAGNOSES—*cont.* :

Ice and ice-creams, 576
 Influenza, 394
 Koch-Weeks bacillus, 520
 Leprosy, 313
 Malaria, 490
 Malignant disease, 512
 Malignant oedema, 405
 Meat, 596
 Milk, 593
 Moulds, 450
 Opsonic index, 192
 Pfeiffer's reaction, 159
 Phagocytosis, 189
 Plague, 380
 Pneumonia, 391
 Protozoa, 475
 in water, 579
 Rabies, 508
 Relapsing fever, 474
 Ringworm, 454
 Russell's bodies, 512
 Sarcoma ventriculi, 226
 Septic diseases, 216
 Sewage, 588
 Shell-fish, 577
 Smegma bacillus, 304, 314
 Soil, 586
 Suppuration, 216
 Syphilis, 534
 Tetanus, 400
 Thrush, 456
 Treponema pallidum, 534
 Trichophytosis, 454
 Trypanosomes, 470
 Tuberculosis, 299
 (milk), 594
 Typhoid bacillus in water, 570
 fever, 345
 Water, 552
 Watercress, 577
 Welch's bacillus, 405
 Xerosis, 273
 Yeasts, 442
 pathogenic, 444
 Exhaustion theory, 184
 Eye-pieces, 125
 FARCIN DES BŒUFS, 431
 Farey, 317
 Favus, 455

Fermentation, 31, 438
 acetic acid, 33
 alcoholic, 32
 bottom, 440
 butyric acid, 33
 lactic acid, 33
 top, 440
 tube, 74
 Ferments, 32
 anti-, 175
 Films, 84
 blood, 87, 490
 interlamellar, 122
 Filters, 42, 577
 Filtration, 42, 577
 as a disinfectant, 602
 sand, 549
 'Finger and toe' disease, 385
 Finkler-Prior Spirillum, 423
 Fixation of complement, test, 165
 Fixing, specimens, 86
 tissues, 77, 512
 by alcohol and ether, 87
 by corrosive sublimate, 77, 512
 Fletcher, 499
 Flagella staining, 105
 Flasks, yeast, 43, 67
 Fleas, 379, 530
 Flies and disease, 339
 preventing access of, 116
 tsetse, 469, 499
 Fluid media, growths in, 60
 Food poisoning, 35, 347, 596
 Foot and mouth disease, 524
 Formalin for disinfecting, 612
 fixing tissues, 77
 preserving cultures, 107
 preserving specimens, 108
 Formate broth, 74, 568
 Fowl enteritis, 381, 422
 Fränkel's pneumococcus, 384
 Frankland's method for air analysis,
 583
 Freezing microtome, 79
 Friedlander's capsule stain, 103
 pneumo-bacillus, 389
 Frozen sections, 78, 99
 Fuchsin agar, 573
 bodies, 510
 carbol, 90
 Fumigation, 607, 612
 Fungus disease, 432, 449
 Fusiform bacillus, 273

MANUAL OF BACTERIOLOGY

- Heat, moist, 599
- Hermann's solution, 512
- Herpes zoster, 524
- Herpetomonas, 466
- Hesse's method for air analysis, 582
- Hot-air sterilizer, 38
- Hydroa gestationis, 524
- Hydrocele fluid culture medium, 53
- Hydrochloric acid, 615
- Hydrogen peroxide, 611
- Hydrophobia, 502
- Hyphae, 446
- Hyphomycetes, 446
 - examination, 450
 - pathogenic, 449
- Ice, organisms in, 547, 576
 - creams, 576
- Identification of organisms, 110
- Illumination, 124
- Immersion lenses, theory of, 127
- Immunity, 177
 - acquired, 178, 183, 184, 186, 188
 - atrophic, 185
 - inherited, 185
- Infection, 177
 - hospital, 201
 - reading, 210, 401, 403
 - of bacillus, 346
 - of action of, 35, 74
 - of. See Culture Media
 - of defecation of, 60
 - of organs, organisms of, 544
 - of violet, anilin, 90
 - of action of blood, &c., 179
 - of es, 602
 - of es, 277, 289, 291, 322, 426
 - of main, 93, 534
 - of cell, 316
 - of cell, 180
 - of anti-bodies, 177
 - of es, 221
 - of diagnosis of, 223
 - of ions in, 222
 - of method, 93
 - of audius's modification, 95
 - of ether's modification, 95
 - of omin, 97
 - of ing of, 97
 - of active cocci, 225
 - of m. ulcerating, 540

- Intravenous inoculation, 114
 Invertase, 32
 Investigation of micro-organisms, 109
 Iodine, 609
 Gram's, 94
 trichloride, 609
 Iodoform, 615
 Irrigation, 119
 Izal, 614
- JENNER's blood stain, 92
 John's disease, 308
- KALA-AZAR, 471
 Kerol, 614
 Klebs-Löffler bacillus, 242
 Koch's 'comma' bacillus, 408
 Koch's postulates, 137
 Koch-Weeks bacillus, 520
- LANKESTERELLA, 497
 Leguminosæ, fixation of nitrogen by, 30
 Leishman-Donovan body, 471
 Lenses, microscopical, 126
 immersion, 127
 Leprosy, 309
 diagnosis of, 313
 Leprosy-like disease of rats, 313
 Leptothrix buccalis, 435
 Leucocytes, migration of, 182
 in milk, 592, 595
 Leucocytozoon canis, 497
 Leuconostoc, 16
 Levaditi's stain, 535
 Life-history, studying, 113, 115
 Life without bacteria, 3
 Light as a disinfectant, 602
 effect of, on bacteria, 21
 Litmus media, 51
 Local Government Board disinfecting solution, 610
 Löffler's methylene blue, 89
 serum, 53
 Loop, standard, 619
 Lustgarten's bacillus, 314, 532
 Lymphadenitis, ovine, 309
 Lymphangitis, 210
 Lysins, 157
 Lysol, 614
- MACROGAMETE, 458
 Macrophages, 183
 Madura disease, 432
 cultivation, 434
 staining, 433
 varieties, 433
 Malachite green media, 326, 573
 Malaria, 479
 diagnosis of, 490
 parasites, 480, 486
 inoculation, 480
 mosquito phase, 483
 varieties, 486
 Malignant disease, 510
 Malignant adenoma, 401
 clinical examination, 405
 Malignant pustule, 234
 Mallein, 323
 in diagnosis, 325
 Malta fever, 524
 Marasmus, 215
 Mastigophora, 465
 Mastoid disease, 526
 McDougall's fluid, 614
 Menstrues, 526
 Measurement, microscopical, 132
 Measures and weights, 621
 Meat, 347, 595
 Media, culture, 46. See Culture
 Media
 Medical antiseptics, 616
 Mediterranean fever, 524
 Membranous rhinitis, 520
 Meningitis, 388, 526
 cerebro-spinal, 218
 posterior basic, 220
 Mercaptan, 35
 Mercuric chloride, 609
 iodide, 610
 Mercury pyogenic, 202
 Merismopedia, definition of, 15
 Metchnikoff's spirillum, 422
 Methylated spirit, 76
 Methylene blue. Löffler's, 89
 borax, 491
 carbolic, 89
 Micrococcus, definition of, 13
 agilis, 597
 bombycia, 499
 candicans, 597
 catarrhalis, 224, 394
 cereus albus, 207
 flavus, 207

MANUAL OF BACTERIOLOGY

- ecus, cinereus*, 220
epidermidis albus, 206
avescens, 207
gonorrhoea, 221
anceolatus, 384
deliensis, 225, 524, 590
meningitidis, 218
coformans, 208, 510
pasteuri, 364
tyogenes, aureus, 204
 albus, 206
 citreus, 206,
 tenuis, 384
tragenus, 225
tyingens, 208
mete, 458
ter, 133
millimetre, 133
 133, 621
ages, 183
aps, bacteriological, 123
eridium, 198
ren Audouini, 451
orfar, 455
ues, 79, 82
r's corpuscles, 499
er
Mucor mucedo, 448
Mucous membranes, organisms of,
 542
Mumps, 526
Mustard oil, 615
Mycelium, 446
Mycetoma, 482
Mycetozoa, 465
Mycoses, 449
Mycosis tonsillaris, 435
Mytilotoxin, 36
Myxomycetes, 465
Myxosporidia, 498

NAGANA, 467
Nasal mucus, germicidal action of,
 543
Nagar, 219
Nastin, 311
Necrosis, 35
Needles, 43, 14
Negri bodies, 503
Neisser's stain, 270
Neuritis, diphtheritic, 250, 251
Nitrification, 26
 stages in, 28

- Organisms and disease, 109, 134, 137
- Organisms, cultivation of, 37
 enumeration of, 71, 198
 identification of, 110
 influence of a mixture of, 19
 isolation of, 37, 68
 of air, water, and soil, 597
 of air-passages, 543
 of conjunctivæ, 542
 of genital tract, 544
 of mouth, 543
 of nose, 543
 of skin, 542
 of stomach and intestine, 544
 of urinary tract, 544
 ultra-microscopic, 131
- Osmic acid fixation, 470, 476, 512
- Osteomyelitis, 205, 210, 330
- Otitis, 214, 527
- Oven, hot-air, 38
- Ozæna, 528
- Ozone, 611
- PAKER'S DISCS, 557
- Pappenheim's solution, 315
- Para-colon bacillus, 327, 348
- Para-dysentery bacillus, 355
- Para-typhoid fever, 348
- Paraffin, embedding in, 80
 sections, 83
 sections, mounting, 100
- Paralysis, diphtheritic, 250, 262
- Paramecium coli, 475
- Parasites, 135
- Parolitis, 526
- Pasteurization of milk, 591
- Pasteur's fluid, 55
- Peat, germicidal action of, 338, 411
- Pébrine, 498
- Pemphigus, 523, 524
- Penicillium glaucum, 448
- Peppermint oil, 615
- Peptone water, 48
- Pericarditis, 222, 387, 531
- Peritonitis, 387, 528
- Permanganate method, 532
- Permanganates, 611
- Pertussis, 529
- Petri dishes, 70
- Petri's method for air analysis, 583
- Petruschky's litmus whey, 360
- Pfeiffer's reaction, 157, 159
- Phagocytes, 183
- Phagocytosis, 182, 188
 estimation of, 189
- Phenol, 612
- Phlebitis, 203
- Phlogosin, 206
- Phosphorescence, 34
- Physiological salt solution, 85
- Picro-carminæ, 87
- Piedra, 455
- Pigment, formation of, 34
- Pink torula, 597
- Pinta, 455
- Pipettes, 44, 46, 193
- Piroplasmata, 494
 cultivation of, 495
- Pitfield's flagella stain, 107
- Pityriasis, 455
- Plague, antiserum, 376
 bacillus of, 367
 diagnosis, 380
 pathogenesis, 371
 spread, 377
 vaccines, 373
- Plasmodium, 479, 480
 Kochii, 490
 malarie, 486
 præcox, 493
 vivax, 487
- Plate bottles, 71
 cultures, 68
 agar, 71
 anaërobic, 73
 gelatin, 69
- Platinum needles, 43
- Plaut's method, 432
- Pleomorphism, 14
- Pleuropneumonia, 131, 384
- Plummer bodies, 510
- Pneumobacillus of Friedländer, 389
- Pneumococcus, Fränkel's, 384
- Pneumo-mycosis, 449
- Pneumonia, 383
 diagnosis, 391
- Poisons, bacterial, 35, 136
 tolerance to, 177
- Porcelain filters, 578
- Postulates, Koch's, 137
- Potassium permanganate, 611, 615
- Potato, 51. See Culture Media
- Precipitins, 176
- Pressure, effect of, on bacteria, 21

MANUAL OF BACTERIOLOGY

- of bacteria, 22
- er-Capaldi media, 359
- bacterial, pyogenic, 202
- xic, 36
- na, 493
- capsulatus hominis, 235
- irabilis, 597
- lgaris, 597. *See* *B. proteus*
- enkeri, 597
- putrefaction, 23
- ta, 9
- , 457
- visions of, 459
- amination of, 475
- in cancer, 510
- variola and vaccinia, 514
- water, 579
- phtheria bacillus, 263
- ation to *B. diphtheriæ*, 267
- tuberculosis, 308
- illus of, 308
- 529
- 85, 351
- osis, 498
- , 35
- fever, 530
- ant. 11
- Reaction, Bordet-Durham, 169, 174
- cholera-red, 336
- Pfeiffer's, 157, 159
- Voges-Proskauer, 364
- Rebipelagar, 569
- Receptors, 179
- chemo-, 177, 185
- Relapses, theory of, 343
- Relapsing fever, spirillum of, 473
- Resolving power, 130
- Retention theory, 185
- Rheumatism, 530
- Rheumatoid arthritis, 531
- Rhinitis, membranous, 250
- atrophic, 528
- Rhinoscleroma, 532
- Ringworm, 450
- cultivation, 452
- examination, 454
- varieties, 450
- Rocking microtome, 82
- Roll cultures, 72
- Romanowsk. stain, 92
- Roup, diphtheritic, 274
- Buffer bodies, 510
- Russell's corpuscles, 510

- Sedgwick and Tucker's method for
 air analysis, 534
 Sedimentation test, 174
 Septic diseases, 200
 tank process, 587
 Septicæmia, 138
 a, 138
 Sera, anti-microbial, 156
 antitoxic, 139
 Serum, culture media of, 52, 53
 germicidal action of, 179
 Sewage, 586
 examination of, 588
 Sewers, air of, 587
 Shake culture, 74
 Shell-fish, examination of, 577
 pathogenic organisms in, 337
 Side-chain theory, 141
 Silica jelly, 29
 Silver salts, 611
 pyogenic, 202
 Skatole, 26
 Skatole-carboxylic acid, 25, 244, 264
 Skin diseases, 522
 organisms of, 205, 206, 542
 Sleeping sickness, 468
 Slides, cleaning, 105
 hollow ground, 120
 Smallpox, 513
 Smear preparations, 87
Smegma bacillus, 313
 staining, 304, 314
 Sodium bisulphate, 576
 Soil, 585
 nitrification in, 26
 Solubilities, 621
 Solutions, normal, 56
 Specimens, preserving pathological,
 108
 Spirilla, 16, 408. *See also* *Vibrio*
 Spirillum, definition of, 16
 cholerae Asiatica, 408
 varieties, 413
 of cholera, isolation from
 water, 575
 of Finkler and Prior, 423
 Metchnikovi, 422
 Obermeieri, 473
 rabrum, 424
 tyrogenum, 424
 Spirochaeta, 16, 472
 Duttoni, 474
 Obermeieri, 473
 pallida, 532
 pertensis, 539
 recurrentis, 473
 refringens, 533
 in cancer, 533
 in ulcers, 533
 in ulcerating granuloma, 540
 in yaws, 539
 Spleen, germicidal substance from,
 180
 in immunity, 187
 Sporangium, 417
 Spore formation, 12
 Spore staining, 104, 442
 Spores, resistance to heat, 13, 18, 599
Sporidium vaccinale, 516
 Sporozoa, 459, 476
 Spruce, 529
 Stage, microscopical, 123
 Staining methods, 89
 cover-glass specimens, 97
 capsules, 103
 flagella, 105
 Gram, 93
 sections, 101
 spores, 104
 Stains, 89
 Standard loop, 619
 Standardization of antitoxin, 257
 of media, 56
Staphylococcus, 16
 species of. *See Micrococcus*
 Steam as a disinfectant, 599
 sterilizer, 39
 Stegomyia, 541
 Sterilization, 37
 discontinuous, 4
 of cotton wool, 45
 of glass vessels, 45
 of milk, 591
 Sterilizer, hot air, 38
 Griffith's, 576
 writer's, for milk, 592
 steam, 39
 Still's *diplococcus*, 220
 Stimulins, 188
 Stomach, organisms of, 544
 Strangles, 211
Streptococcus, definition of, 15
 diagnostic table, 214
 anginosus, 213
 brevis, 212
 conglomeratus, 211, 212, 501

MANUAL OF BACTERIOLOGY

- Coccus*, equi, 214
- Crysipelatis*, 210, 219
- Fecalis*, 213
- Longus*, 212
- Medius*, 212
- Pyogenes*, 209
 - anti-serum, 211
 - in milk, 487
- Rheumaticus*, 214, 531, 532
- Salivarius*, 213
- Scarlatinae*, 211, 212, 501
- Strix* infections, 426
- Strix*, acid-fast, 277
- Actinomyces*, 428
- Eppingeri*, 431
- Leproides*, 311
- Madura*, 434
- Trichae*, 425
- age condenser, 124
- , resolution of, 20
- arous acid, 607
- arsenisation, 149
- Bakker, 200
- clinical examination, 216
- conditions modifying, 203
- due to chemical agents, 201
- influence of dose, 204
- , resolution of, 204
- Thermal death point, determination of, 501
- Thermophilic bacteria, 18, 535
- Thionin, carbol, 90
- Thrush, 455
- Ticks, 474, 496
- Tinea, 450
- Tinfoil, 44
- Tissue-fibrinogen, 182
- Tissues, preparation of, 76
- Tolerance to poisons, 177
- Torula, pink, 597
- Torulae, 437
- Toxins, 36, 136, 145
- Toxoids, 150, 257
- Toxones, 150, 258
- Trachoma, 538
- Treatment, antiseptic, 616
 - antitoxic, 148
- Treponema pallidum*, 533
- Trichomonas, 466
 - species of, 474
- Trichophyton, 450
 - megalosporon ectothrix, 451
 - endothrix, 451
- Trypanoplasma, 466
- Trypanosoma, 466
 - , resolution of, 204

- Tuberculosis, 277
 anatomy of, 277
 avian, 290
 complement fixation in, 299
 diagnosis of, 299 *et seq.*
 disinfection in, 298
 in the horse, 291
 mammalian, 291
 occurrence of, 288, 295
 pseudo, 308
 Royal Commissions on, 292
 spread of, 294
 Tuberculous food, 296, 297
 sputum, staining, 299
 tissues, staining, 302
 urine, 304
 Tulase, 286
 Turpentine, pyogenic, 202
 Twort's stain, 463
 Typhoid bacillus, 328
 in the blood, 330
 in milk, 590, 594
 in water, 336, 570
 survival of, 334, 335
 isolation from stools, 330, 346
 vaccine, 342
 Typhoid fever, 327
 agglutination reaction, 332, 345
 and oysters, 337
 and sewer gas, 340
 diagnosis of, 345
 in animals, 330
 Typhus fever, 539
 Tyrotoxin, 36, 590

 ULCERATING granuloma, 540
 Ulcerative endocarditis. *See* Endocarditis, infective
 Ulcers, 424
 Ultra-microscopic organisms, 131
 Units, antitoxin, 256, 259
 Unna's method, 102
 Urea, fermentation of, 34
 Urinary operations, infection in, 362
 organs, organisms of, 544
 Urine, colon bacillus in, 362
 smegma, bacillus in, 304, 314
 tubercle bacillus in, 304
 typhoid bacillus in, 330
 Ushinsky's fluid, 55

 VACCINES, 197 (also under individual organisms)
 Anthrax, 237
 Cholera, 418
 Plague, 373
 Typhoid, 342
 Vaccinia, 517
 Vaccinia, 513
 Van Ermengem's flagella stain, 105
 Varicella, 539
 Variola, 513
 Vibrio, definition of, 16, 409
 cholera, 408
 Berolinensis, 414
 Danubicus, 414
 Deneke, 424
 El Tor, 414
 Elwers, 413
 Finkler, 423
 Ivanoff, 414
 Massowah, 414
 Metchnikovi, 422
 Sanarelli, 413
 Vibrios of mouth, 424
 Vincent's angina, 272
 Virulence, to increase, 112
 Visibility, limit of, 131
 Vitality of cultures, 112
 Voges-Proskauer reaction, 364

 WATER, bacteriology of, 546
 number of organisms in, 547
 effect of sand filtration, 549, 551
 effect of sedimentation, 549
 effect of storage, 548, 550
 bacteriological analysis of, 552
 pathogenic organisms in, 570, 575
 colon bacillus in, 559, 563, 567
 comma bacillus in, 411, 575
 sterilization of, 576
 sterilizers, 576
 typhoid bacillus in, 396
 Weigert's method, 96
 Weights and measures, 621
 Whooping cough, 529
 Widal reaction, 170, 172
 Wooden tongue, 428
 Wool-sorter's disease, 234
 Wright's capsule, 193







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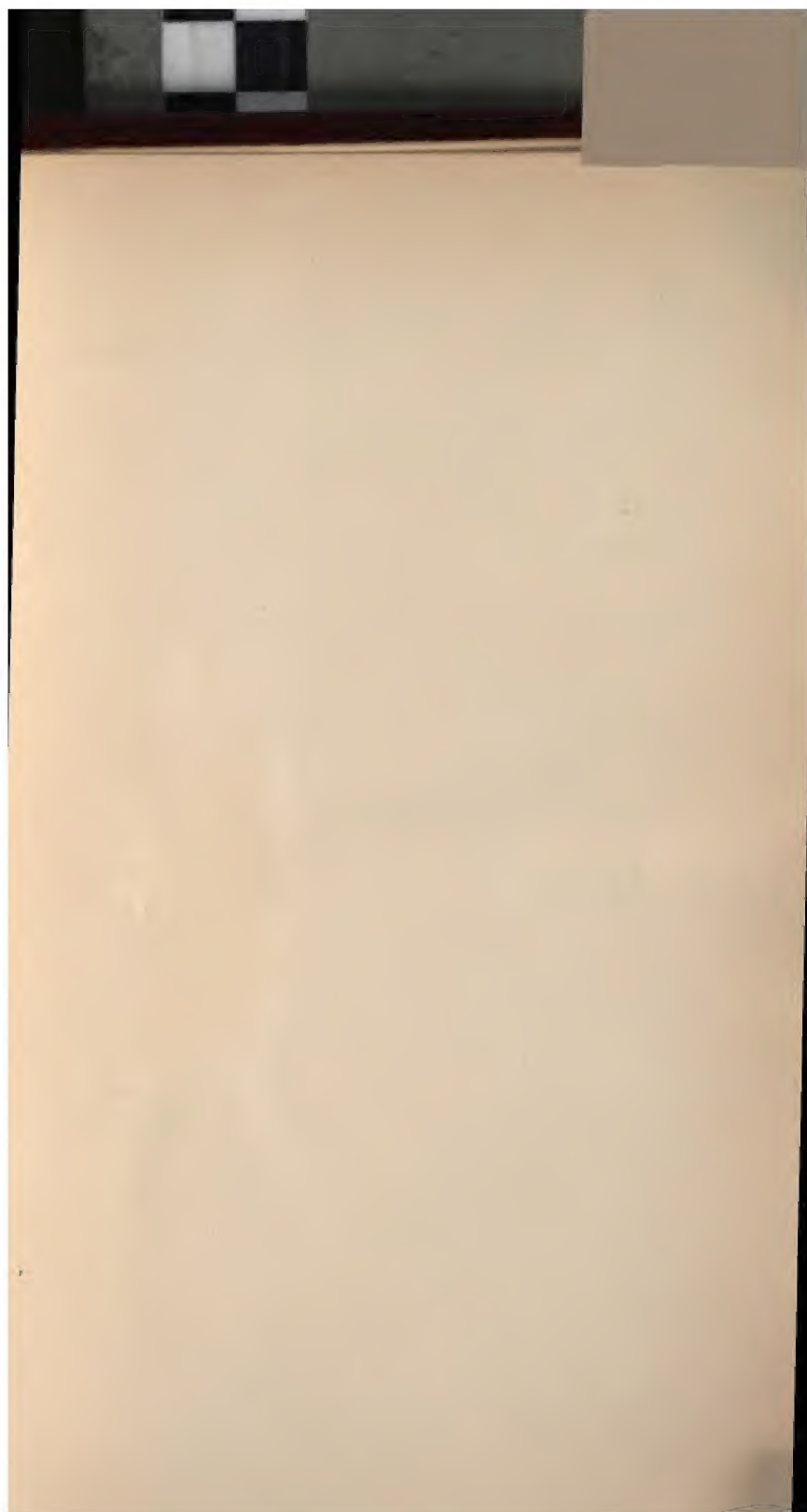
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